Supporting information

Chimeric peptidomimetics of SOCS 3 able to interact with JAK2 as anti-inflammatory compounds.

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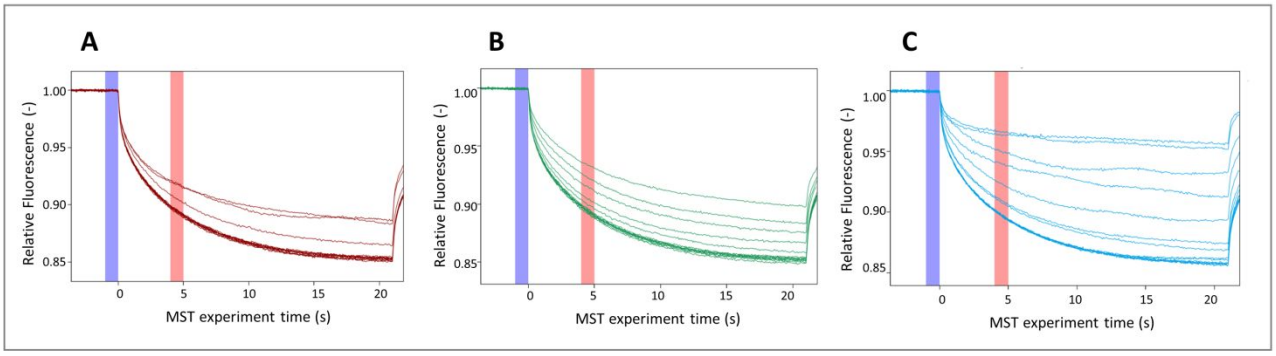


Figure S1. Thermophoretic traces of MST assays for the binding to JAK2 of (**A**) ESSCONG, (**B**) restKIRESSCONG, (**C**) KIRCONG chim peptides.

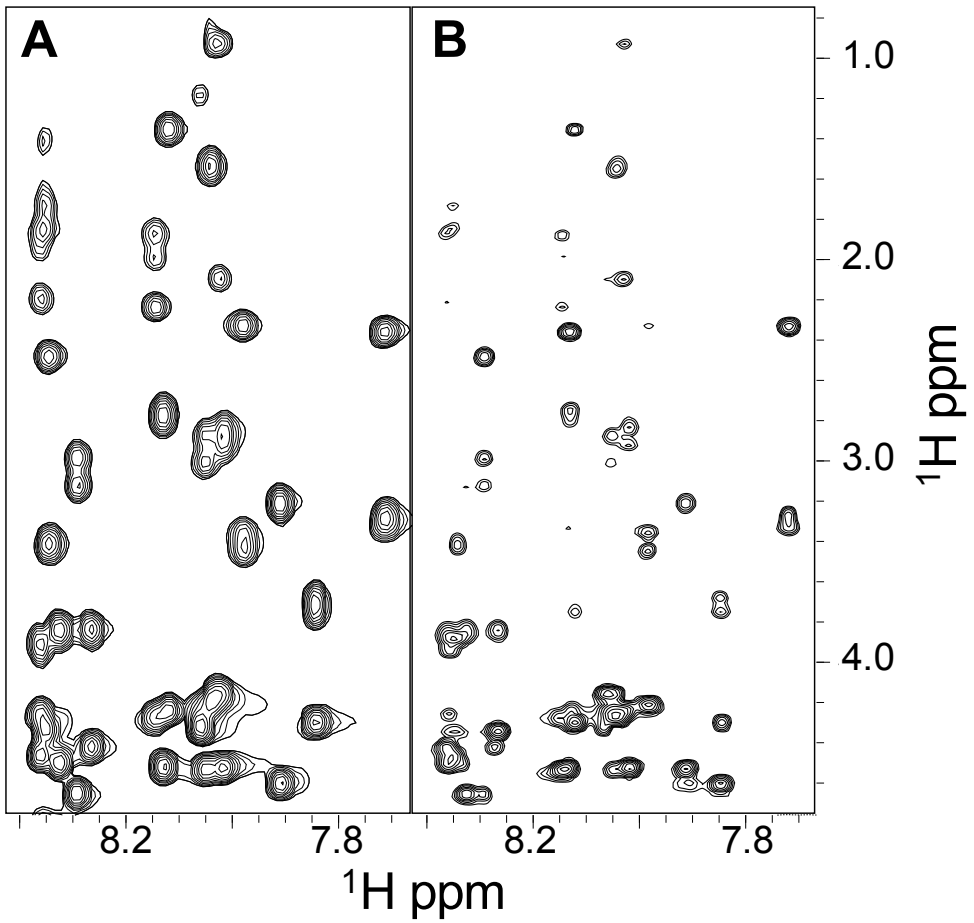


Figure S2. 2D [^{1}H-^{1}H] TOCSY (A) and ROESY 250 (B) spectra of KIRCONG chim peptide in H_2O. Peptide concentration was 770 μM. The figure shows spectral regions containing H_N/aliphatic protons correlations.

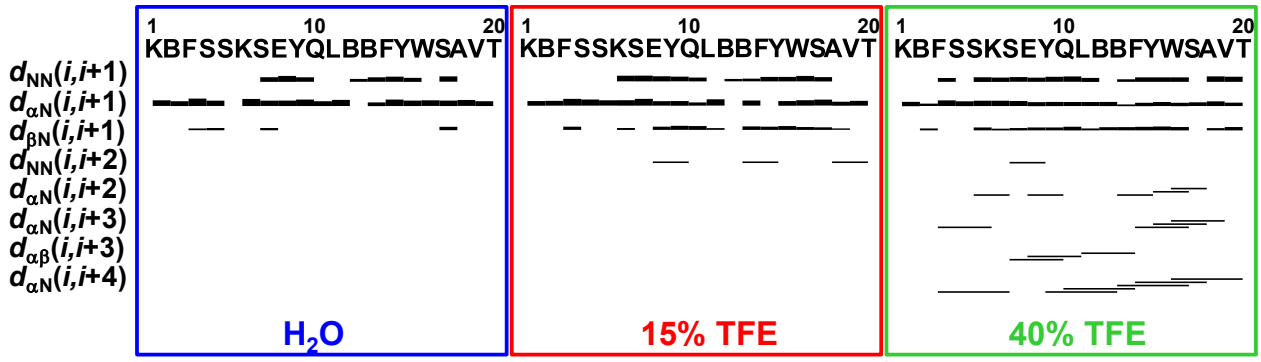


Figure S3. ROE pattern in H_2O (enclosed in the blue square) obtained from analysis of the ROESY 250 spectrum of the KIRCONG chim peptide; NOE patterns of KIRCONG chim obtained from analyses of NOESY 300 spectra acquired in 15% TFE and 40% TFE (highlighted within red and green squares, respectively); “dxy(i,i+z)” denotes a cross-peak between "x" and "y" protons in residues “i” and “i+z”, respectively; the thickness of the lines is proportional to the distance: shorter distances are represented by thicker bars. The amino acid sequence is shown on top with the one letter code; B indicates the β-Ala residue.

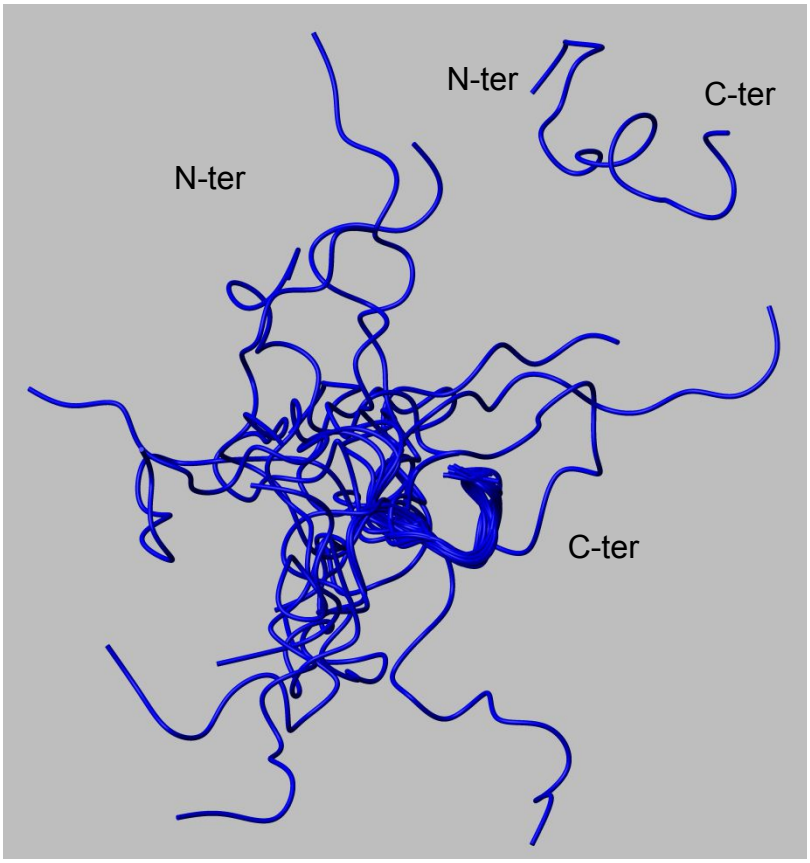


Figure S4. NMR structure of KIRCONG chim peptide calculated in H_2O: the best 20 conformers are superimposed on the backbone atoms of residues 14-20 (RMSD=1.11 Å). The first conformer of the NMR ensemble is also shown in the upper right corner. Structure calculations included 77 upper distance limits (38 intraresidue, 36 short-range, and 3 medium-range) and 91 angular constraints.

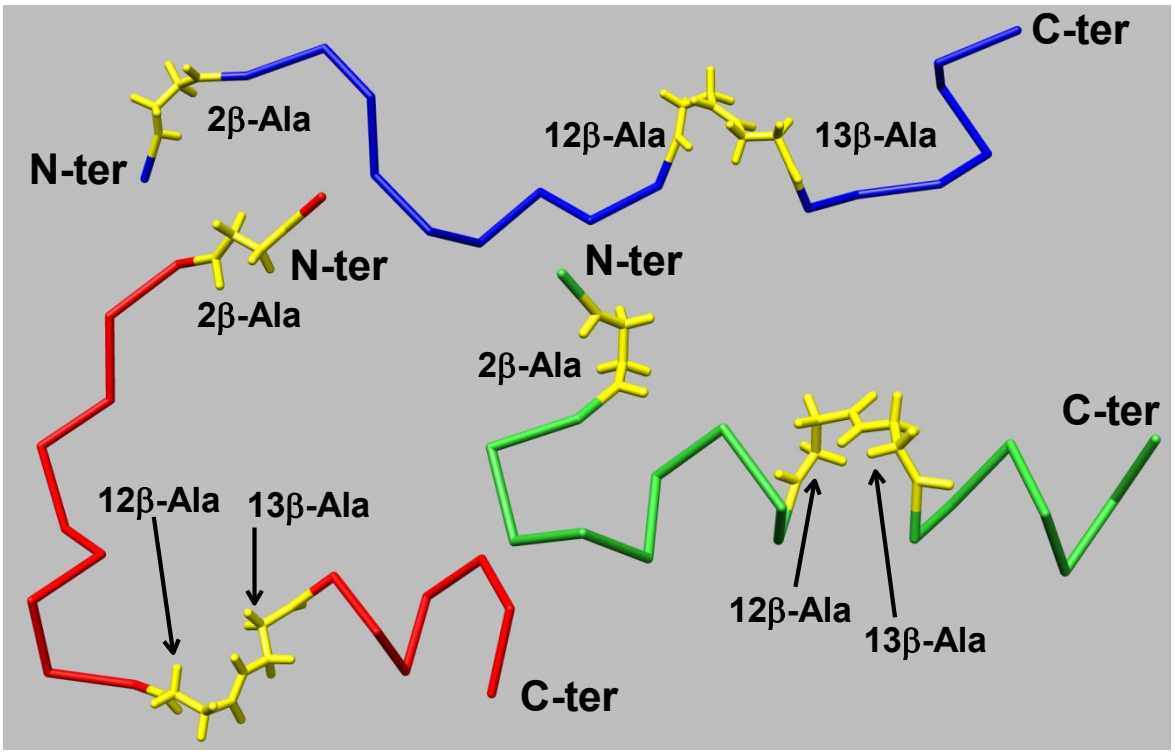


Figure S5. First conformers of KIRCONG chim peptide NMR ensembles in H_2O (blue), H_2O/TFE (85/15, v/v, red), and H_2O/TFE (60/40, v/v, green). The structures are reported in the Cα trace mode with all backbone and side chains atoms of the β-alanines shown and colored yellow.

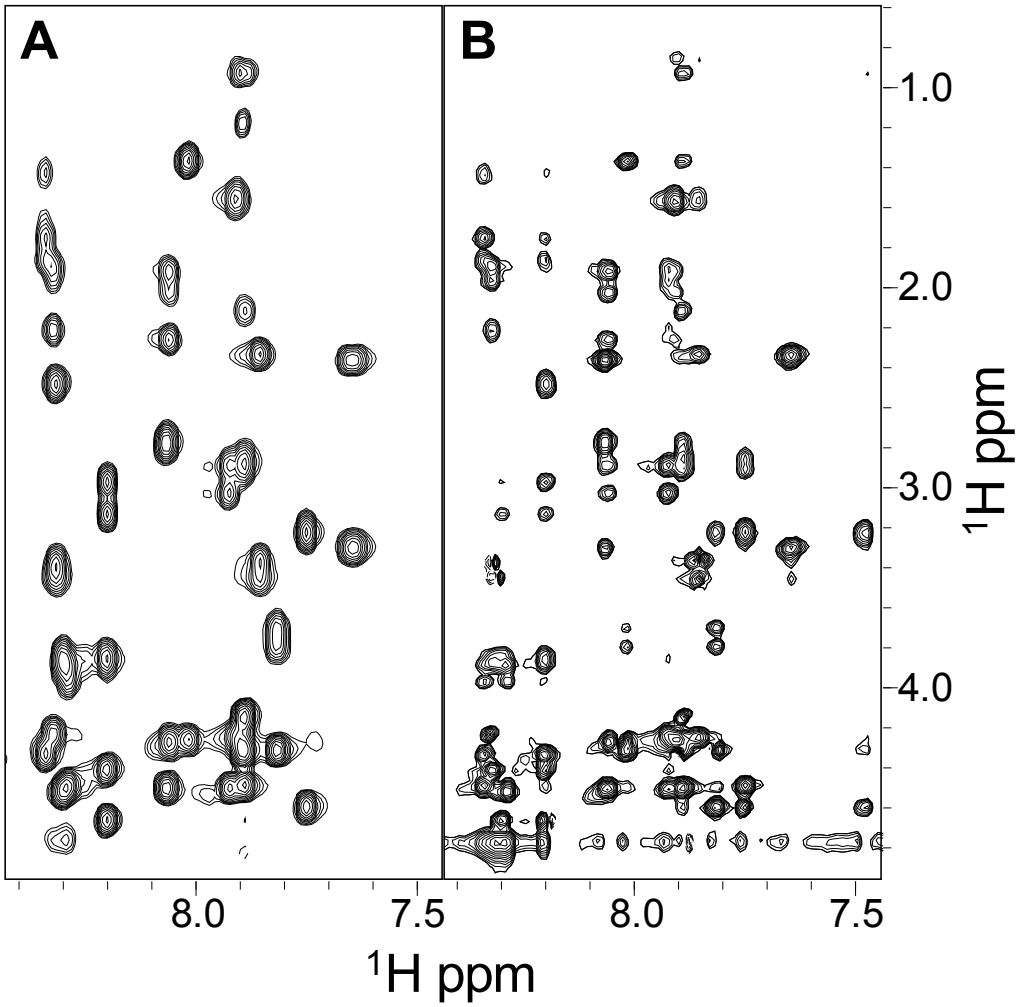


Figure S6. 2D [^{1}H-^{1}H] TOCSY (A) and NOESY 300 (B) spectra of KIRCONG chim peptide in H_2O/TFE (85/15, v/v). Peptide concentration was 770 μM. The regions of the spectra containing H_N/Hα and side chains correlations are shown.

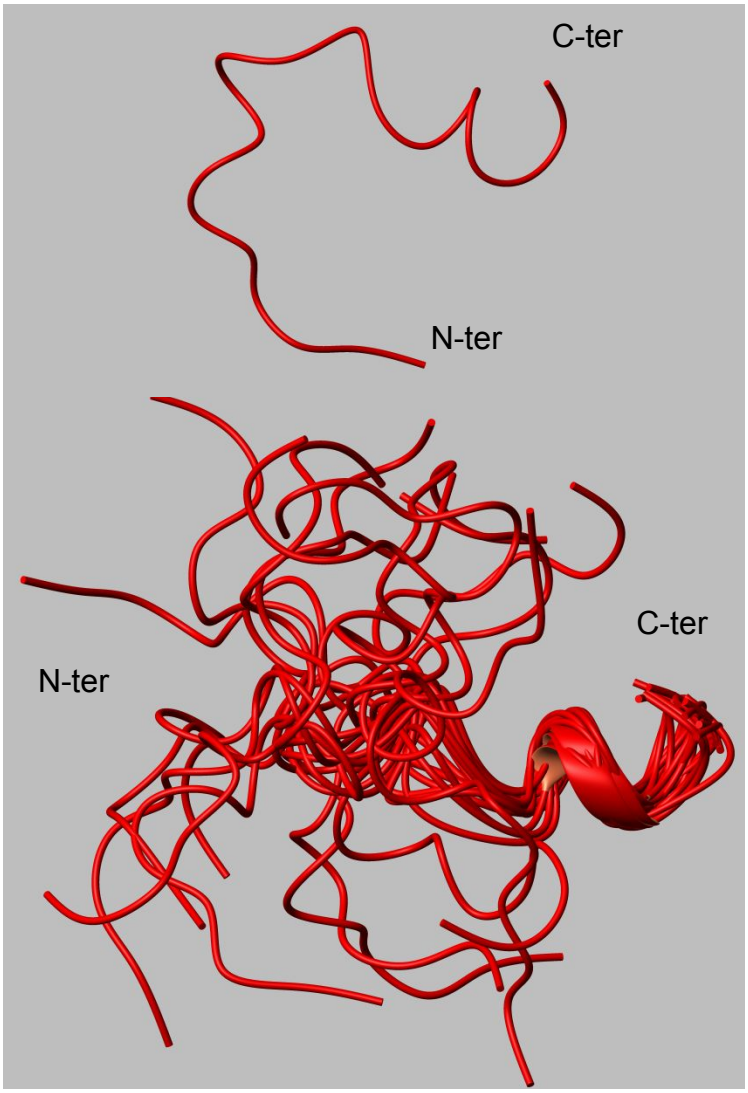


Figure S7. NMR structure of KIRCONG chim peptide in H_2O/TFE (85/15, v/v): the best 20 conformers are superimposed on the backbone N,C,O,CA atoms of residues from 10 to 20 and the CB atoms of the β-Ala residues 12 and 13 (RMSD=1.93 Å). The first conformer of the NMR ensemble is shown in the upper panel. The NMR structure was calculated from 121 upper distance limits (60 intraresidue, 58 short-range, and 3 medium-range) and 79 angle constraints.

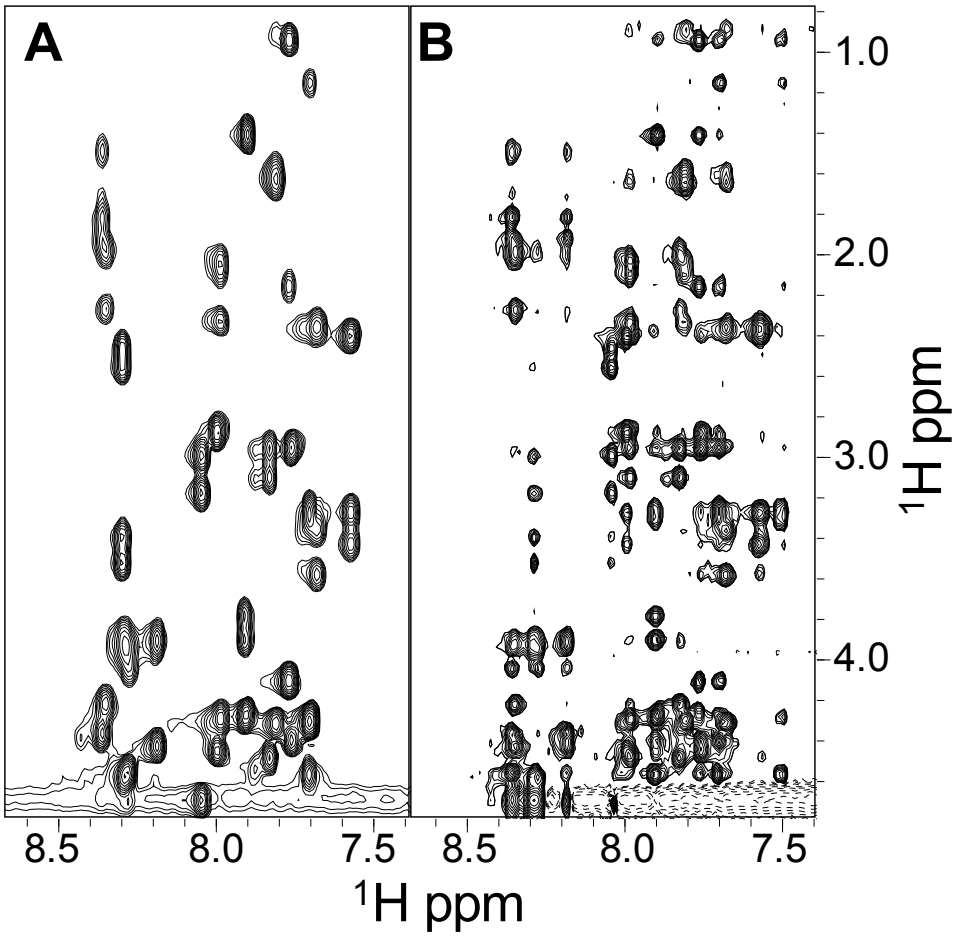
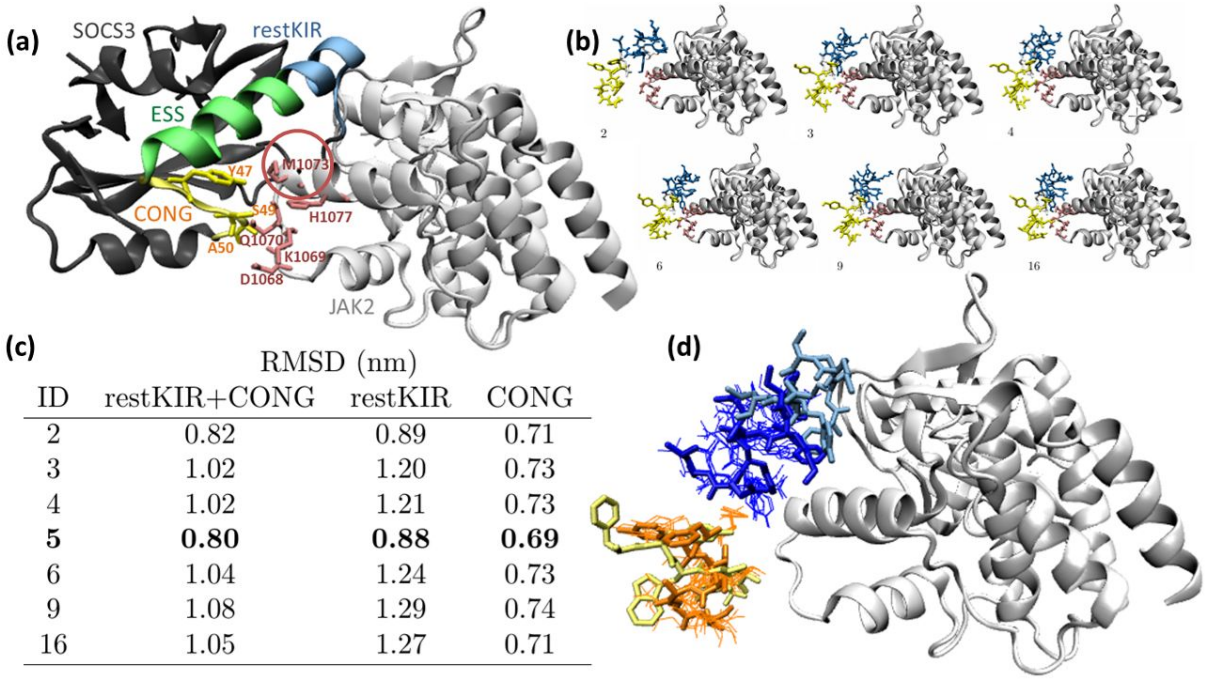


Figure S8. 2D [^{1}H-^{1}H] TOCSY (A) and NOESY 300 (B) spectra of KIRCONG chim peptide in H_2O/TFE (60/40, v/v). Peptide concentration was 770 μM. Only the spectral regions containing H_N/aliphatic protons correlations are shown.



(c)

	RMSD (nm)		
ID	restKIR+CONG	restKIR	CONG
2	0.82	0.89	0.71
3	1.02	1.20	0.73
4	1.02	1.21	0.73
5	**0.80**	**0.88**	**0.69**
6	1.04	1.24	0.73
9	1.08	1.29	0.74
16	1.05	1.27	0.71

Figure S9. (a) SOCS3/JAK2 (PDB): 4GL9 [1], chains A and E) with the fragment restKIRESSCONG and residues described in the text highlighted, and the centre of the docking box subsequently chosen on JAK2 also highlighted (red circle) and (b) selected putative poses of KIRCONG chim docked to JAK2, colour code: JAK2 (white), SOCS3 (gray), restKIR (blue), ESS(green), CONG (yellow). (c) Root mean squared deviation of the restKIR and CONG heavy atom fragments with respect to the same atoms in SOCS3, and (d) their overlap where the optimum pose (ID5 with RMSD=0.80) represented in orange/dark blue and the SOCS3 fragment yellow/blue are highlighted for comparison.

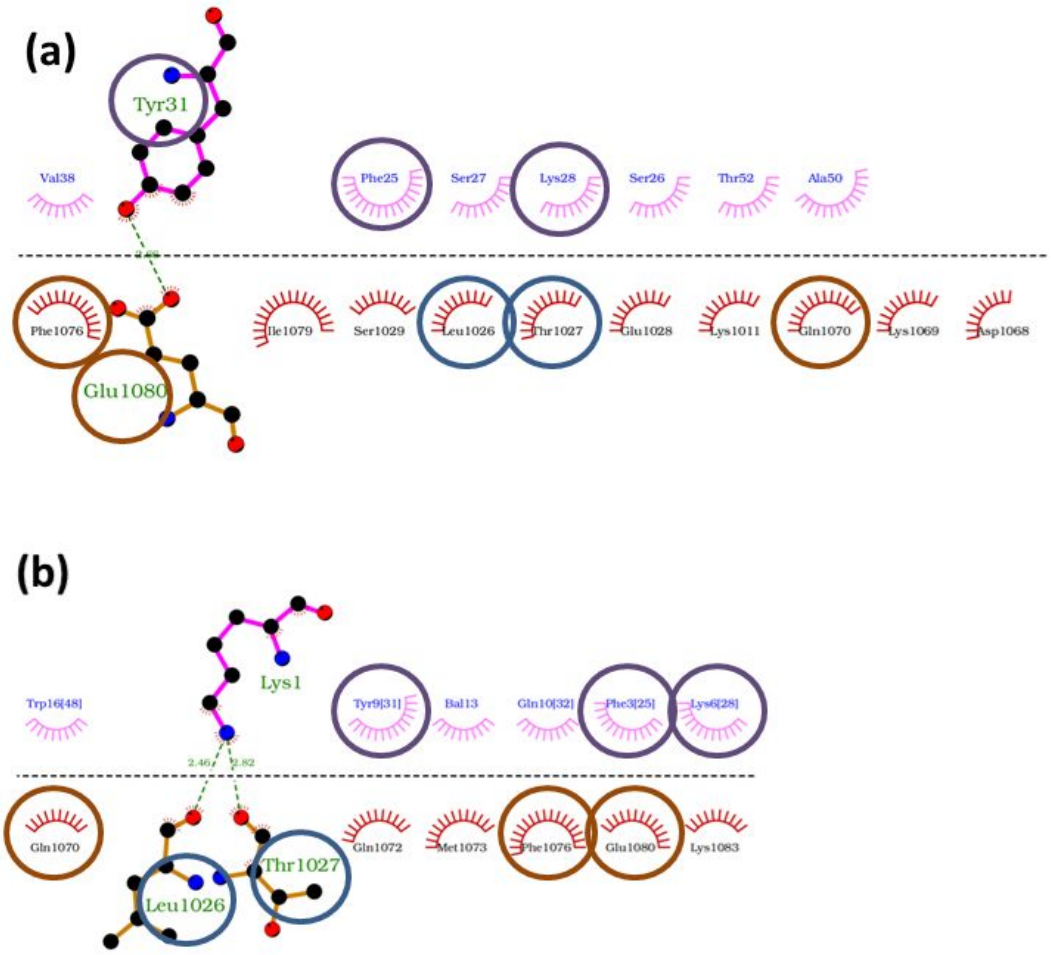


Figure S10. Schematic diagram of (a) the interaction between restKIRESSCONG and JAK2 (configurations extracted from PDB: 4GL9 [1], chains A and E), and (b) KIRCONG chim docked to JAK2. Interacting peptides residues are highlighted in pink, while JACK residues are highlighted in red, residues forming hydrogen bonds are green. Hydrogen bonds distances measured in nm are also indicated. On the peptides analogous interacting residues are circled in purple. On JAK2, residues interacting with both peptides are circled (blue if interacting with KIR and brown when interacting with CONG).

Table S1. ^{1}H chemical shifts of KIRCONG chim peptide in H_2O at pH=4.2 and T=25°C.

Residue	HN	Hα	Hβ	Hγ	Others
1K	8,35	3,87	1,82	1,37	Hδ 1,66 Hε 2,95
2BAL	8,34	2,47	3,39		
3F	8,29	4,62	2,99–3,12		Hδ 7,25 Hε 7,30 Hζ 7,25
4S	8,32	4,48	3,83		
5S	8,36	4,44	3,90		
6K	8,35	4,33	1,82	1,41	Hδ 1,67 Hε 2,94
7S	8,26	4,39	3,81		
8E	8,36	4,24	1,86–1,95	2,18	
9Y	8,05	4,51	2,86–2,99		Hδ 7,03 Hε 6,77
10Q	8,14	4,26	1,87–1,98	2,22	Hε 6,82–7,44
11L	8,04	4,18	1,55	1,55	δCH3 0,86–0,93
12BAL	7,97	2,33	3,41		
13BAL	7,72	2,35	3,28		
14F	8,13	4,50	2,73–2,77		Hδ 7,08 Hε 7,25
15Y	8,02	4,51	2,85–2,89		Hδ 7,00 Hε 6,76
16W	7,91	4,58	3,17–3,21		Hε1 10,0 Hδ1 7,19 Hη2 7,22 Hε3 7,51 Hζ3 7,15 Hζ2 7,40
17S	7,85	4,28	3,66–3,71		
18A	8,12	4,22	1,34		
19V	8,03	4,14	2,08	0,93–0,94	
20T	8,06	4,31	4,22	1,19	

Table S2. Structure statistics of KIRCONG chim peptide NMR structure bundle in H_2O.

Residual target function, Å²	0.14±0.05
Residual NOE violations	2
Number ≥ 0.1 Å*	1
Residual angle violations	0
Atomic pairwise RMSD, Å	
Backbone atoms (all residues)	4.09±0.86
Heavy atoms (all residues)	5.30±0.79
Procheck analysis (all residues)#	
Residues in core regions	18.4%
Residues in allowed regions	63.9%
Residues in generous regions	11.6%
Residues in disallowed regions	6.1%

* CYANA [2] average violations
#PROCHECK_NMR [3] statistics

Table S3. ^{1}H chemical shifts of KIRCONG chim peptide in H_2O/TFE (85/15, v/v) at pH=4.2 and T=25°C.

Residue	HN	Hα	Hβ	Hγ	Others
1K	8,35	3,87	1,82	1,37	Hδ 1,66 Hε 2,95
2BAL	8,34	2,47	3,39		
3F	8,29	4,62	2,99–3,12		Hδ 7,25 Hε 7,30 Hζ 7,25
4S	8,32	4,48	3,83		
5S	8,36	4,44	3,90		
6K	8,35	4,33	1,82	1,41	Hδ 1,67 Hε 2,94
7S	8,26	4,39	3,81		
8E	8,36	4,24	1,86–1,95	2,18	
9Y	8,05	4,51	2,86–2,99		Hδ 7,03 Hε 6,77
10Q	8,14	4,26	1,87–1,98	2,22	Hε 6,82–7,44
11L	8,04	4,18	1,55	1,55	δCH3 0,86–0,93
12BAL	7,97	2,33	3,41		
13BAL	7,72	2,35	3,28		
14F	8,13	4,50	2,73–2,77		Hδ 7,08 Hε 7,25
15Y	8,02	4,51	2,85–2,89		Hδ 7,00 Hε 6,76
16W	7,91	4,58	3,17–3,21		Hε1 10,0 Hδ1 7,19 Hη2 7,22 Hε3 7,51 Hζ3 7,15 Hζ2 7,40
17S	7,85	4,28	3,66–3,71		
18A	8,12	4,22	1,34		
19V	8,03	4,14	2,08	0,93–0,94	
20T	8,06	4,31	4,22	1,19	

Table S4. Structure statistics of KIRCONG chim peptide NMR ensemble in H_2O/TFE (85/15, v/v).

Residual target function, Å²	0.20±0.01
Residual NOE violations	2
Number ≥ 0.1 Å*	1
Residual angle violations	0
Atomic pairwise RMSD, Å	
Backbone atoms (all residues)	3.91±0.72
Heavy atoms (all residues)	5.19±0.72
Procheck analysis (all residues)#	
Residues in core regions	47.4%
Residues in allowed regions	40.8%
Residues in generous regions	7.6%
Residues in disallowed regions	4.2%

* CYANA [2] average violations
#PROCHECK_NMR [3] statistics

Table S5. ^{1}H chemical shifts of KIRCONG chim peptide in H_2O/TFE (60/40, v/v) at pH=4.33 and T=25°C.

Residue	HN	Hα	Hβ	Hγ	Others
1K		3,91	1,89	1,44	Hδ 1,73 Hε 3,03
2BAL	8,30	2,48–2,56	3,40–3,51		
3F	8,05	4,68	2,98–3,18		Hδ 7,26 Hε 7,34
4S	8,29	4,57	3,88–3,92		
5S	8,27	4,54	3,94–4,03		
6K	8,36	4,36	1,92	1,49	Hδ 1,82 Hε 3,03
7S	8,19	4,41	3,88–3,91		
8E	8,35	4,21	1,95–2,00	2,27	
9Y	7,83	4,47	2,96–3,10		Hδ 7,10 Hε 6,83
10Q	7,99	4,28	2,02–2,08		Hε 6,69–7,35
11L	7,82	4,29	1,66	1,61	δCH3 0,91–0,95
12BAL	7,68	2,35–2,37	3,35–3,59		
13BAL	7,58	2,37–2,40	3,27–3,43		
14F	7,99	4,43	2,89		Hδ 7,29 Hε 7,14
15Y	7,76	4,39	2,96–2,98		Hδ 6,92 Hε 6,74
16W	7,71	4,56	3,29–3,31		Hε1 9,84 Hδ1 7,15 Hη2 7,15 Hε3 7,51 Hζ3 7,13 Hζ2 7,41
17S	7,91	4,26	3,78–3,91		
18A	7,90	4,25	1,42		
19V	7,77	4,10	2,15	0,94–0,97	
20T	7,70	4,29	4,26	1,16	

Table S6. Structure statistics of KIRCONG chim peptide NMR ensemble in H_2O/TFE (60/40, v/v).

Residual target function, Å²	0.06±0.04
Residual NOE violations	1
Number ≥ 0.1 Å*	0
Residual angle violations	0
Atomic pairwise RMSD, Å	
Backbone atoms (all residues)	0.72±0.22
Heavy atoms (all residues)	1.25±0.28
Procheck analysis (all residues)#	
Residues in core regions	62.9%
Residues in allowed regions	28.7%
Residues in generous regions	7.4%
Residues in disallowed regions	1.1%

* CYANA [2] average violations
#PROCHECK_NMR [3] statistics

Table S7. List of H-bonds calculated with UCSF-Chimera[4] for all 20 representative KIRCONG chim NMR conformers calculated in H_2O/TFE (60/40, v/v). UCSF-Chimera default parameters (i.e., constraints relaxed by 0.4 angstroms and 20 degrees) were used for assessing the presence of a H-bond[4].

Conformer	Acceptor	Donor
Model 1	GLU 8 OE2	SER 4 N
	TYR 9 O	β-Ala 13 N
	GLN 10 O	PHE 14 N
	β-Ala 13 O	SER 17 N
	PHE 14 O	ALA 18 N
	TRP 16 O	VAL 19 N
	TRP 16 O	THR 20 N
Model 2	GLN 10 O	β-Ala 12 N
	TYR 9 O	β-Ala 13 N
	GLN 10 O	PHE 14 N
	β-Ala 13 O	SER 17 N
	PHE 14 O	ALA 18 N
	TRP 16 O	VAL 19 N
	TRP 16 O	THR 20 N
	TRP 16 O	CON2 21 N
Model 3	GLN 10 O	β-Ala 12 N
	TYR 9 O	β-Ala 13 N
	GLN 10 O	PHE 14 N
	β-Ala 13 O	SER 17 N
	PHE 14 O	ALA 18 N
	TRP 16 O	VAL 19 N
	TRP 16 O	THR 20 N
Model 4	GLN 10 O	β-Ala 12 N
	TYR 9 O	β–Ala 13 N
	GLN 10 O	PHE 14 N
	β-Ala 13 O	SER 17 N
	PHE 14 O	ALA 18 N
	TRP 16 O	VAL 19 N
	TRP 16 O	THR 20 N
	TRP 16 O	CON2 21 N
Model 5	LYS 6 O	SER 7 OG
	LYS 6 O	GLN 10 NE2
	GLN 10 O	β-Ala 12 N
	TYR 9 O	β-Ala 13 N
	GLN 10 O	PHE 14 N
	β-Ala 13 O	SER 17 N
	TRP 16 O	VAL 19 N
	TRP 16 O	THR 20 N
Model 6	TYR 9 O	β-Ala 13 N
	GLN 10 O	PHE 14 N
	β-Ala 13 O	SER 17 N
	TRP 16 O	VAL 19 N

	TRP 16 O	THR 20 N
Model 7	GLU 8 O	β-Ala 12 N
	TYR 9 O	β-Ala 13 N
	GLN 10 O	PHE 14 N
	β-Ala 13 O	SER 17 N
	TYR 15 O	ALA 18 N
	TRP 16 O	VAL 19 N
	TRP 16 O	THR 20 N
	SER 17 O	CON2 21 N
Model 8	TYR 9 O	β-Ala 13 N
	GLN 10 O	PHE 14 N
	β-Ala 13 O	SER 17 N
	TRP 16 O	VAL 19 N
	TRP 16 O	THR 20 N
Model 9	GLU 8 OE1	SER 4 N
	GLU 8 O	β-Ala 12 N
	TYR 9 O	β-Ala 13 N
	GLN 10 O	PHE 14 N
	β-Ala 13 O	SER 17 N
	TRP 16 O	VAL 19 N
	TRP 16 O	THR 20 N
Model 10	TYR 9 O	β-Ala 13 N
	GLN 10 O	PHE 14 N
	β-Ala 13 O	SER 17 N
	TRP 16 O	VAL 19 N
	TRP 16 O	THR 20 N
Model 11	GLN 10 O	β-Ala 12 N
	TYR 9 O	β-Ala 13 N
	GLN 10 O	PHE 14 N
	β-Ala 13 O	SER 17 N
	TRP 16 O	VAL 19 N
	TRP 16 O	THR 20 N
Model 12	GLN 10 O	β-Ala 12 N
	TYR 9 O	β-Ala 13 N
	GLN 10 O	PHE 14 N
	β-Ala 13 O	SER 17 N
	TRP 16 O	VAL 19 N
	TRP 16 O	THR 20 N
Model 13	TYR 9 O	β-Ala 12 N
	TYR 9 O	β-Ala 13 N
	β-Ala 13 O	SER 17 N
	PHE 14 O	ALA 18 N
	TYR 15 O	ALA 18 N
	TRP 16 O	THR 20 N
Model 14	LYS 6 O	SER 7 OG
	TYR 9 O	β-Ala 13 N
	GLN 10 O	PHE 14 N
	β-Ala 13 O	SER 17 N

	TRP 16 O	THR 20 N
Model 15	SER 4 O	SER 5 OG
	GLN 10 O	β-Ala 12 N
	TYR 9 O	β-Ala 13 N
	GLN 10 O	PHE 14 N
	β-Ala 13 O	SER 17 N
	TRP 16 O	THR 20 N
	VAL 19 O	THR 20 N
Model 16	GLU 8 O	β-Ala 12 N
	TYR 9 O	β-Ala 13 N
	GLN 10 O	PHE 14 N
	β-Ala 13 O	SER 17 N
	PHE 14 O	ALA 18 N
	TRP 16 O	THR 20 N
	SER 17 O	CON2 21 N
Model 17	GLN 10 O	β-Ala 12 N
	TYR 9 O	β-Ala 13 N
	GLN 10 O	PHE 14 N
	β-Ala 13 O	SER 17 N
	PHE 14 O	ALA 18 N
	TYR 15 O	ALA 18 N
	TYR 15 O	VAL 19 N
	TRP 16 O	THR 20 N
Model 18	GLN 10 O	β-Ala 12 N
	TYR 9 O	β-Ala 13 N
	GLN 10 O	PHE 14 N
	β-Ala 13 O	SER 17 N
	PHE 14 O	ALA 18 N
	TRP 16 O	THR 20 N
Model 19	SER 4 O	SER 5 OG
	TYR 9 O	β-Ala 13 N
	GLN 10 O	PHE 14 N
	β-Ala 13 O	SER 17 N
	PHE 14 O	ALA 18 N
	TRP 16 O	THR 20 N
	TRP 16 O	CON2 21 N
Model 20	SER 7 O	LEU 11 N
	TYR 9 O	β-Ala 13 N
	GLN 10 O	PHE 14 N
	β-Ala 13 O	SER 17 N
	PHE 14 O	ALA 18 N
	TYR 15 O	ALA 18 N
	TYR 15 O	VAL 19 N
	TRP 16 O	THR 20 N

Peptide Synthesis: Solid phase peptide synthesis and purification were performed as already reported [5]. Briefly SOCS3 derived peptides reported in Table 1 were synthesized employing the solid phase method on a 50 μmol scale following standard Fmoc strategies of SPPS [6]. They were purified through RP-HPLC and identified as already reported [7]. To allow cellular assays, the fragment 48–60 of the HIV Tat protein carrying TAMRA as a fluorophore was conjugated to KIRESS, KIRCONG chim and control peptide (NC) (Table 1) in a stepwise manner. Purified peptides were lyophilized and stored at −20°C until use.

Microscale Thermophoresis: MST experiments were carried out with a Monolith NT 115 system (Nano Temper Technologies) equipped with 100% LED and 40% IR-laser power. Labeling of His-tagged Catalytic Domain of JAK2 (residues 826-1132) (Carna Biosciences) was achieved with the His-Tag labeling Kit RED-tris-NTA, as already reported [8]. SOCS3 derived peptides were used in the following concentrations: ESSCONG 404μM, KIRCONG chim 409μM, restKIRESSCONG 408μM in labeling buffer (Nano Temper Technologies); the dye concentration was adjusted to 100 nM while the protein concentration was 200nM. Subsequently, 100μL of protein and 100μL of dye were incubated in the dark for 30 min. To monitor binding of SOCS3 derived peptides, a serial dilution (1:1) [9]was carried out by preparing 14-16 samples on average. Premium capillaries were employed for analysis, at 25°C in 50mM Tris-HCl, 150mM NaCl, 0.05% Brij35, 1mM DTT, 10% glycerol, 15% TFE buffer at pH 7.5. An equation implemented by the software MO-S002 MO Affinity Analysis, provided by the manufacturer, was used for fitting data at different concentrations.

Circular Dichroism (CD) Spectroscopy: CD spectroscopy experiments were carried out by employing a Jasco J-810 spectropolarimeter (JASCO Corp, Milan, Italy) at room temperature in the spectral range 190-260 nm and spectra are averaged over two scans, to which blanks were subtracted. CD signals were converted to mean residue ellipticity with deg* cm^2*$dmol^{-1}$*res^{-1} as units. Scan speed value was 20 nm/min, band width 2.0 nm, resolution 0.2 nm, sensitivity 50 mdeg and response 4 sec. Peptides concentration was 50 μM in a quartz cuvette with a path-length of 0.1 cm [10, 11]. Spectra were acquired in 10 mM phosphate buffer at pH 7.4 in mixtures with TFE (2,2,2 trifluoroethanol)[12].

NMR studies: NMR spectra of the KIRCONG chim peptide (as N-term amine free variant) were recorded at 25 °C on a Varian Unity Inova 600 MHz spectrometer provided with a cold probe. NMR samples were prepared by dissolving the peptide (0.9 mg) either in H_2O with 10% v/v D_2O (98% D, Sigma-Aldrich, Milan, Italy), or in aqueous mixtures containing 15% and 40% v/v 2,2,2-trifluoroethanol-D3 (99.5% isotopic purity, Sigma-Aldrich, Milan, Italy); with final sample

volumes equal to 500 µL, for a final peptide concentration of 770 µM. To conduct NMR structural analyses the following 2D [^{1}H, ^{1}H] experiments were recorded: TOCSY (Total Correlation Spectroscopy)[13], NOESY (Nuclear Overhauser Enhancement Spectroscopy)[14], ROESY (Rotating Frame Overhauser Enhancement Spectroscopy)[15] and DQFCOSY (Double Quantum-Filtered Correlated Spectroscopy)[16]. Typical acquisition parameters included 16-64 scans, 128-256 FIDs in t1, 1024 or 2048 data points in t2. Mixing times for TOCSY experiments were set to 70 ms whereas, a mixing time equal to 300 ms was used to record NOESY spectra; ROESY experiments were acquired with a mixing time equal to 250 ms. Residual water signal was suppressed through excitation sculpting[17]. A standard strategy was followed to gain proton resonance assignments[18]. Trimethylsilyl-3-propionic acid sodium salt-D4 (TSP) (99% D, Armar Scientific, Switzerland) was used as internal standard for chemical shifts referencing. The Varian software VNMRJ 1.1D (Varian/Agilent Technologies, Milan, Italy) was implemented to process NMR spectra that were next analyzed with the program NEASY[19] included in CARA (Computer Aided Resonance Assignment).

NMR Structure calculations and analysis: NMR solution structures of KIRCONG chim peptide in H_2O and H_2O/TFE mixtures were calculated with the software CYANA (version 2.1)[2]. The non-native amino acid β-Alanine was built and added to the CYANA[2] standard residue library. Distance constraints (i.e., upper distance limits) were generated from manual integration of peaks in 2D [^{1}H, ^{1}H] NOESY spectra (300 ms mixing time) or 2D [^{1}H, ^{1}H] ROESY spectrum for the sample in H_2O; the GRIDSEARCH module of CYANA software was implemented to obtain angular constraints[2]. 100 random conformers were initially generated and in the end the 20 structures provided with the lowest CYANA target functions and better obeying to experimental constraints, were selected as representative NMR conformers and additionally analyzed with the software MOLMOL[20] and UCSF Chimera[4], that were also used to generate structure images.

Computational Methods for protein preparation: JAK2 (PDB: 4GL9 [1], chain A) missing atoms and residues were reconstructed with Swiss Model[21]. The JAK2 was then minimized by performing a steepest descent minimization to be stopped either when the maximum force was lower than 1000.0 kJ/mol/nm or when 50000 minimization steps were performed with 0.005 kJ/mol energy step size, with Verlet cutoff scheme, short-range electrostatic cut-off and Van der Waals cut-off of 1.0nm. We used GROMOS 54a7 force field[22] and run the minimization as implemented in the Gromacs package v. 2016.1 [23].

Computational Methods for peptide preparation and docking: 20 ligand structures obtained by NMR were docked (rigid docking) to the SOCS3 binding site on JAK2 with AutoDock [24]. Each

system was prepared with AutoDock tools. The docking cubic box had 30.0Å side on all directions and centered on CA of Met1073. Grid spacing was 0.375Å. Each docking based on Lamarckian Genetic Algorithm was performed with 200 runs and 2,500,000 maximum numbers of evaluations and standard parameters. For each starting structure the representative pose of most populated docking cluster was chosen for subsequent analysis. RMSD were calculated on heavy atoms (C,N,O,S) of standard amino acids. 2D ligand-protein interaction diagrams were generated with LigPlot+[25].

Serum stability: Peptides were analysed in diluted fetal bovine serum (FBS) at 25% (w/v) as previously described [26], at a concentration of 80 μM. At the following time points: 0, 0.5, 1, 2 and 4 h, 80 μL aliquots of the incubated solutions were mixed with 80 μL of 15% trichloroacetic acid (TCA) and in order to precipitate serum proteins, were incubated at 2 °C for at least 15 min. Upon a centrifugation (13,000 rpm for 10 min) the supernatant was recovered. Experiments were carried out in triplicate. Reverse phase high performance liquid chromatography (RP-HPLC) was performed on a Shimadzu HPLC equipped with UV detector using a C18-Kinetek column from Phenomenex (Milan, Italy), by employing a gradient from 10 to 80 % of B (acetonitrile 0.1 % TFA) versus A (water 0.1 % TFA) in 20 min.

Cell cultures: VSMCs from mouse aorta were isolated by enzymatic digestion, cultured in Dulbecco´s Modified Eagle Medium (DMEM) supplemented with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine (Sigma-Aldrich) and used between the 4ndand 7th passages. The murine macrophage cell line RAW 264.7 (TIB-71; American Type Culture Collection) was cultured in DMEM with 10% FBS, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine (Sigma-Aldrich).

Cells were made quiescent by 24h incubation in medium with 0% FBS, and then were pre-treated for 90 min with peptides (KIRESS, KIRCONG chim and negative control (NC) conjugated to Tat derived cell penetrating sequence,12.5μM) before stimulation for 1h with recombinant cytokines (IFNγ 10^3 U/mL plus IL-6 10^2 U/mL; PeproTech).

mRNA expression analysis: The total RNA was extracted from cultured cells using TRIzol (Life Technologies) . Target gene expression (*Cxcl10,Ccl5*and *Nox2*) was analyzed in duplicate by real-time quantitative PCR (Applied Biosystem) and normalized to housekeeping gene 18S.

Immunofluorescence studies: VSMCs seeded on glass coverslips were treated for 90min with TAMRA-conjugated peptides before stimulation, then fixed(4% paraformaldehyde), permeabilized (0.5% Triton X-100) and incubated with P-STAT3 antibody (Cell Signaling), followed by fluorescein isothiocyanate-conjugated secondary antibody (Sigma-Aldrich) and nuclear

counterstaining with diamidino-2-phenylindole (Dapi). A confocal fluorescent microscope (Leica) was used to capture images.

Statistical analysis: Results are presented as individual values and mean±SEMof 3 independent experiments. Statistical analysis was performed using Prism 5 (GraphPad Software Inc) and P-value<0.05 was considered significant (ANOVA with Bonferroni's post-hoc test).

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