Supplementary Information

Supplementary table 1: Tryptic Peptides Observed by LCMS Eluting from C4 Column between 13 and 16 minutes (Fractions 7 to 12).

HPLC Fraction	Observed Mass [M](av)[1]	Proposed Structure			Calculated Mass [M](av)[2]
7	**9637.0**	DVSEYSCRELHFTRYVTDGPCRSAKPVTELVCSGQCGPAR	4 -SS-	PSGPDFRCIPDRYRAQRVQLLCPGGEAPRARKVRLVASCKCKRLTR	9637.096
8	**6596.0**	DVSEYSCRELHFTR -SS- PSGPDFRCIPDRYR	knot	SAKPVTELVCSGQCGPAR -SS- (x2) LVASCKCKRLTR	6595.584
	6224.8	DVSEYSCRELHFTR -SS- PSGPDFRCIPDRYR	knot	SAKPVTELVCSGQCGPAR -SS- (x2) LVASCKCKR	6225.132
9	**10165.4**	DVSEYSCRELHFTRYVTDGPCRSAKPVTELVCSGQCGPAR	4 -SS-	WWRPSGPDFRCIPDRYRAQRVQLLCPGGEAPRARKVRLVASCKCKRLTR	10165.760
	6309.0	DVSEYSCRELHFTR -SS- PSGPDFRCIPDRYR	knot	PVTELVCSGQCGPAR -SS- (x2) LVASCKCKRLTR	6309.253
	5938.1	DVSEYSCRELHFTR -SS- PSGPDFRCIPDRYR	knot	PVTELVCSGQCGPAR -SS- (x2) LVASCKCKR	5938.801
10	**7123.2**	DVSEYSCRELHFTR -SS- WWRPSGPDFRCIPDRYR	knot	SAKPVTELVCSGQCGPAR -SS- (x2) LVASCKCKRLTR	7124.198
11	**6753.0**	DVSEYSCRELHFTR -SS- WWRPSGPDFRCIPDRYR	knot	SAKPVTELVCSGQCGPAR -SS- (x2) LVASCKCKR	6753.746
	6836.9	DVSEYSCRELHFTR -SS- WWRPSGPDFRCIPDRYR	knot	PVTELVCSGQCGPAR -SS- (x2) LVASCKCKRLTR	6837.867
12	**6468.1**	DVSEYSCRELHFTR -SS- WWRPSGPDFRCIPDRYR	knot	PVTELVCSGQCGPAR -SS- (x2) LVASCKCKR	6467.415
	2148.0	YVTDGPCR	1 -SS-	VQLLCPGGEAPR	2147.465

[1] Mass from MaxEnt deconvolution of spectral envelope

[2] Average sequence masses calculated using Expasy Compute PI/Mw tool (http://ca.expasy.org/tools/pi_tool.html)

Bold indicates most intense ion in fraction

Supplementary table 2: Tryptic Peptides observed in the C4 fractions by MALDI analysis.

Fraction No.	Observed mass	Proposed Sequence	Position	Sequence mass
1	521.253	TMNR	33-36	521.25059
2	none			
3	1002.52	FHNQSELK	151-158	1002.5009
	1673.817	AENGGRPPHHPFETK	37-51	1673.8148
4	1673.8622	AENGGRPPHHPFETK	37-51	1673.8148
5	1674.8674	AENGGRPPHHPFETK *	37-51	1673.8148
	866.3982	GRKPRPR	170-176	866.5437
6	1219.5352	DFGTEAARPQK	159-169	1219.6071
7	922.4968	CIPDRYR	112-118	922.4569
8	1741.8022	DVSEYSCRELHFTR	52-65	1741.7968
	1678.8192	PSGPDFRCIPDRYR	105-118	1678.81235
	1802.896	SAKPVTELVCSGQCGPAR	74-91	1802.8893
	1007.5525	LVASCKCKR	139-147	1007.5494
9	1741.8016	DVSEYSCRELHFTR	52-65	1741.7968
10	853.5282	LLPNAIGR	92-99	853.526
	1741.7983	DVSEYSCRELHFTR	52-65	1741.7968
11	1035.4959	GQGWQAFKN	1-9	1035.50118
	921.4504	GQGWQAFK	1-8	921.4583
	1057.4802	GQGWQAFKN + Na^+	1-9	
	854.5515	LLPNAIGR *	92-99	853.52593
12	910.4105	YVTDGPCR	66-73	910.4093
	1239.6569	VQLLCPGGEAPR	122-133	1239.652
	921.4433	GQGWQAFK	1-8	921.4583
	2147.1208	YVTDGPCR -ss- VQLLCPGGEAPR	66-73 -ss- 122-143	2147.465
13	none			
14	1037.532	GQGWQAFKN * [1]	1-9	1035.50118
	2594.276	DATEIIPELGEYPEPPPELENNK [1]	10-32	2594.2513

*Deamidated Asn residues
[1] Unexpected non-tryptic cleavage

Expression and purification of sclerostin

Uniformly ^{15}N and $^{15}N/^{13}C$ labelled sclerostin was produced from cells grown in minimal medium containing 1 g/l ^{15}N ammonium sulphate and if appropriate 2 g/l ^{13}C D-Glucose as the sole nitrogen and carbon sources. To improve the quality of the $^{13}C/^{1}H$ HSQC-NOESY spectra, $^{15}N/^{13}C$ labelled samples of sclerostin were prepared that contained unlabelled aromatic residues (Phe, Tyr, Trp and His), which was achieved by the addition of 50 mg/l of the unlabelled amino acids to the minimal medium.

Minimal medium was inoculated at an OD_{600} of 0.1 from an overnight culture and grown at 37^{o}C to an OD_{600} of 0.6-0.8 before inducing protein expression with 10 μM IPTG. Induced cultures were grown overnight at 17^{o}C before cells were harvested by centrifugation. The cell pellet from 6 litres of culture was re-suspended in 100 ml of ice cold PBS with 1 mM EDTA and protease inhibitors (Boehringer). The cells were lysed in a French Press cell disruptor at 20,000 psi, and the soluble fraction clarified by centrifugation at 48,000 g. The volume of the soluble fraction was made up to 1 L with PBS, containing 1 mM EDTA and loaded onto a 100 ml Amylose-HP (New England Biolabs) affinity column and the MBP-Scl step eluted with 10 mM maltose in PBS, containing 1 mM EDTA. Eluted material was diluted 1 in 4 in 25 mM Tris-HCl pH 8.0 and loaded onto a 20 ml Q-HP (GE Healthcare) ion exchange column. The MBP-Scl was eluted with a 0-300 mM NaCl gradient over 20 column volumes. Fractions containing pure MBP-Scl were identified by SDS-PAGE and pooled together. The maltose binding protein was cleaved from sclerostin by addition of TEV protease (~1 mg TEV; 10 mg MBP-Scl) for 5 hours at 25^{o}C. The cleaved material was diluted 1 in 3 in 25 mM sodium phosphate buffer pH 7.0 and loaded onto a 10 ml SP-HP ion exchange column. Sclerostin was eluted by a 0-1.0 M NaCl gradient over 20 column volumes. Fractions containing pure sclerostin were identified by SDS-PAGE, pooled, concentrated to 0.5 mM and buffer-exchanged into 25 mM sodium phosphate buffer, 100 mM NaCl, 10 uM EDTA, 0.01 % (w/v) NaN_3, pH 5.5 for NMR analysis.