

SUPPLEMENTARY MATERIAL

Supplemental Figure 1

1: R I L G A T S L G N **M** Q R A W I L L T L
 AGGATCCTAGGAGCCACATCCCTGGGAATATGCAGCGCGGTGGATTCTGCTCACCTTG
 1: -----!-----!-----!-----!-----!-----!-----!-----!-----!-----!
 TCCTAGGATCCTCGGTAGGGACCCCTTATACGTGCGCGCACCTAAGACGAGTGGAAC 60

1: G L M A C V S A E T R T E L T S D Y P Y
 GGCTTGTATGGCCTGTGTGTCGGCAGAGACGAGAAACAGAGCTGACATCCGATATCCATAT
 1: -----!-----!-----!-----!-----!-----!-----!-----!
 CCGAACTACCGGACACACAGGGCTCTCTGCTCTTGTCTCGACTGTAGGCTAATAGGTATA 120

HA epitope insertion

1: **D** V P D Y A K D **M** Y L D N S S I E E A S
 GACGTGCCAGACTATGCTAAGGATATGTACCTTGACAATAGCTCCATTGAGGAAGCTTCA
 121: -----!-----!-----!-----!-----!-----!-----!-----!-----!-----!
 CTGCAACGGTCTGATAAGATTCTATACATGGAACGTGTTATCGAGGTAACCTTCGAAGT 180

1: G V Y P I D D D D Y S S A S G S G A D E
 GGAGTATATCCTATTGATGATGATGACTATTCTCTGCCTCAGGCTCAGGAGCTGATGAA
 181: -----!-----!-----!-----!-----!-----!-----!-----!-----!-----!
 CCTCATATAGGATAACTACTACTGATAAGAAGACGGAGTCGGAGTCCTCGACTACTT 240

1: D I E S P V L T T S Q L I P R I P L T S
 GACATAGAGAGTCAGTCTGACAAACATCCAACTGATTCCAAGAACATCCAACTCACTAGT
 241: -----!-----!-----!-----!-----!-----!-----!-----!-----!
 CTGTATCTCTCAGGTCAAGACTGTTGAGGTTGACTAAGGTTCTAGGGTGAGTGATCA 300

1: A A S P K V E T **M** T L K T Q S I T P A Q
 GCTGCTTCCCCAAAGTGGAAACCATGACCTTGAGACACAAAGCATTACACCTGCTCAG
 301: -----!-----!-----!-----!-----!-----!-----!-----!-----!
 CGACGAAGGGGTTTACACCTTGGTACTGCAACTCTGTGTTCGTAATGTGGACGAGTC 360

1: T E S P E E T D K E E V D I S E A E E K
 ACTGAGTCACCTGAAGAAACTGACAAGGAGGAAGTGACATTCTGAGGCAGAACAGAGAAG
 361: -----!-----!-----!-----!-----!-----!-----!-----!-----!
 TGACTCAGTGGACTCTTGACTGTTCCCTCAACTGTAAAGACTCCGTCTCTTC 420

1: **L** G P A I K S T D V Y T E K H S D N L F *
 CTGGGCCCTGCTATAAAAGCACAGATGTGTACACGGAGAAACATTGACAAATCTGTTT**TAA**
 421: -----!-----!-----!-----!-----!-----!-----!-----!-----!
 GACCCGGGACGATATTTCTGCTACACATGTGCCTCTTGTAAGTCTGTTAGACAAA 480

1: K R T E V L A A V I A G G G V I G F L F A
 AAACGGACAGAAAGTCTAGCAGCGCTATTGCTGGTGTGATCGGCTTCTCTTGCC
 481: -----!-----!-----!-----!-----!-----!-----!-----!-----!
 TTGCGCTGCTCAAGATCGTCGGAGAACGACCACACTAGCCGAAAGAACCG 540

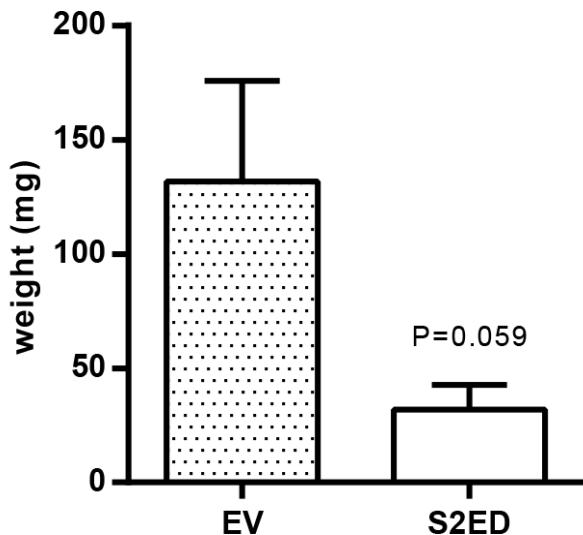
1: I F L I L L V Y R **M** R K K D E G S Y D
 ATTTCTCATCCTGCTATTGGTGTACCGCATCGGAAGAAAGATGAAGGAAGCTACGAC
 541: -----!-----!-----!-----!-----!-----!-----!-----!
 TAAAAGGAGTAGGAGCATAACCATGGCGTACGCCCTCTTCTACTTCCTCGATGCTG 600

1: L G E R K P S S A A Y Q K A P T K E F Y
 CTTGGAGAACGCAAACCATCCAGCGCAGCTTACCGAGAACGACCAACTAAGGAGTTTAT
 601: -----!-----!-----!-----!-----!-----!-----!-----!
 GAACCTCTTGGCTTGGTAGGTGCGCTCGAATGGCTTCCGTGGGTGATTCTCAAAATA 660

1: A * G S
 GCATAAGGATCC
 661: -----!--- 672
 CGTATTCCCTAGG

Murine syndecan-2 cDNA with HA tag insertional mutation (white text, red background).
 Syndecan coding sequence for eFLS2 is in bold and sequence encoding eS2ED is underlined
 with the addition of a stop codon (red).

1 **Supplemental Figure 2**



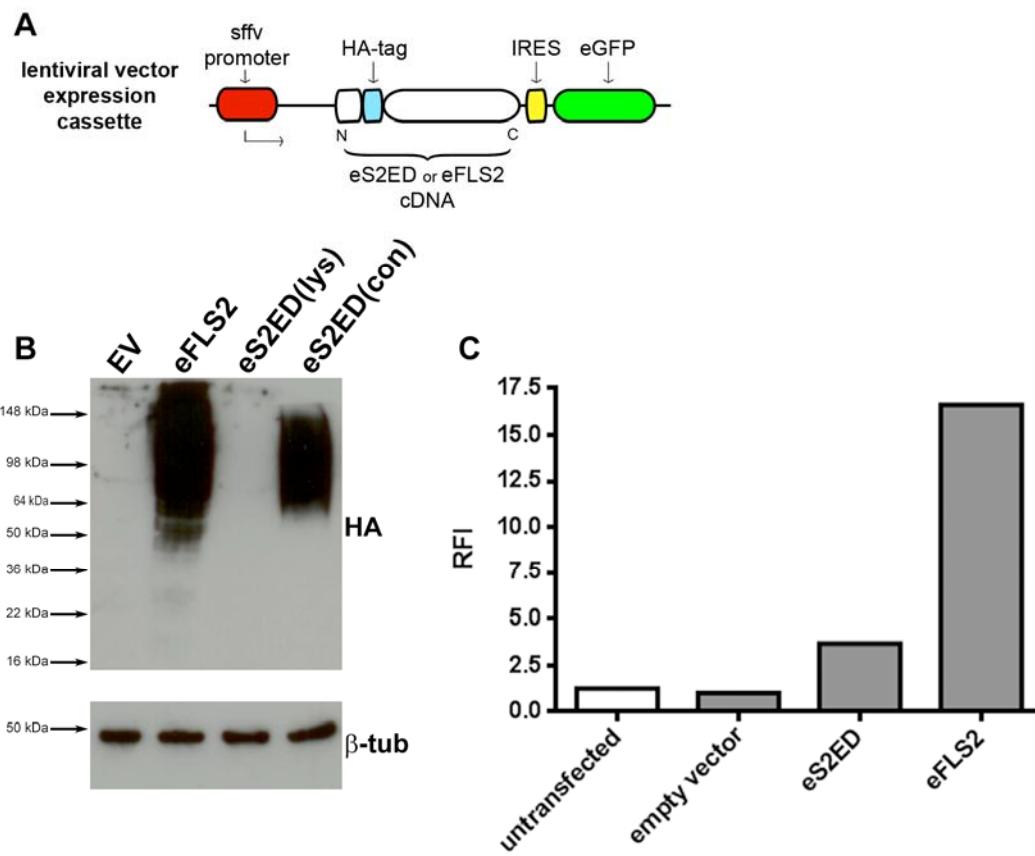
2

3

4 **Tumours derived from eS2ED cells have smaller diameter and mass.** Error bars represent
5 the SEM and significance was calculated using a Students t test.

6

2

1 **Supplemental Figure 3**

2

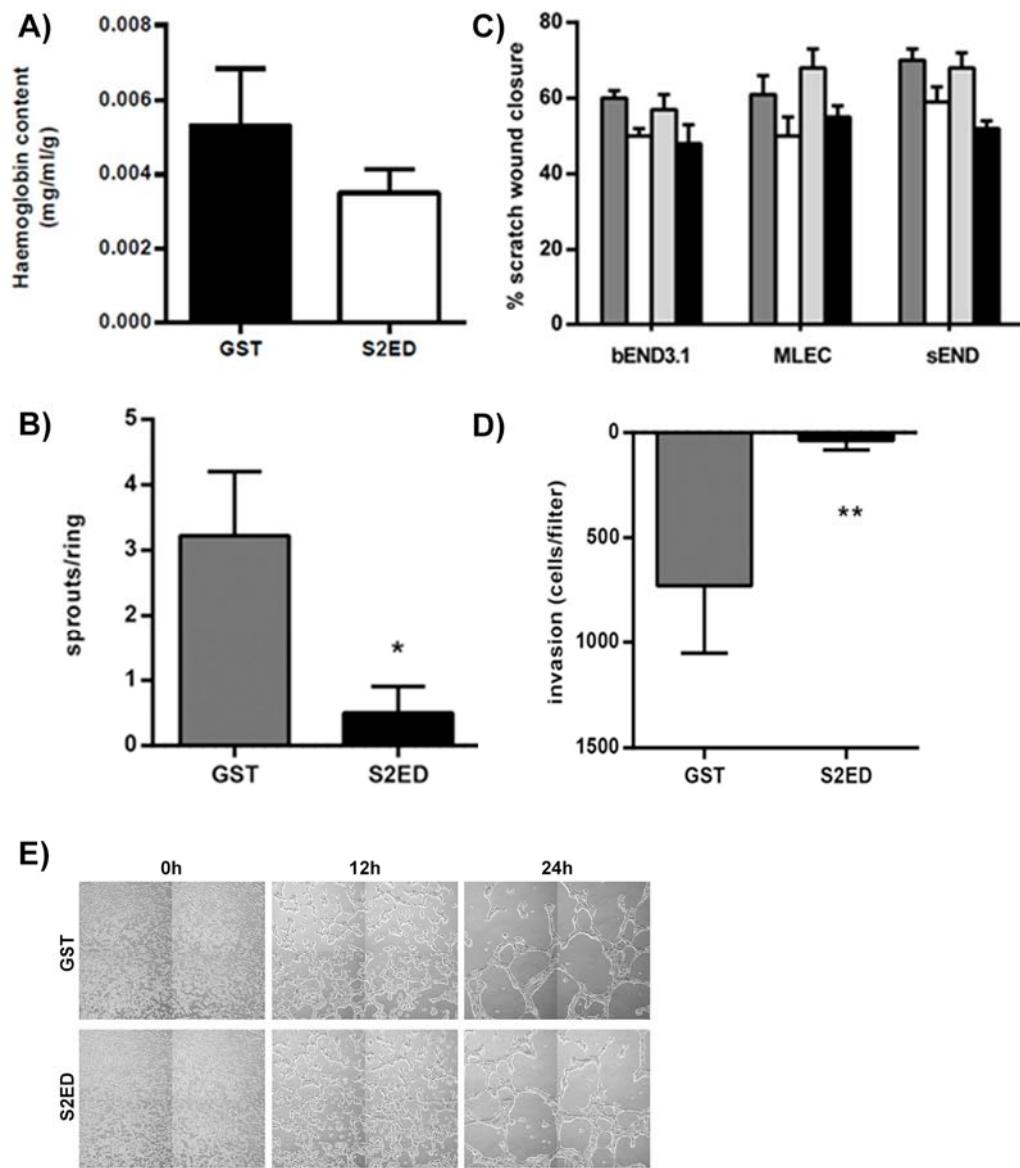
3

4 **Characterisation of the syndecan-2 expressing HEK293t cell lines.** (A) Schematic
 5 diagram of the lentiviral expression cassette used for transfection. (B) Expression of full
 6 length syndecan-2 was demonstrated by western blot using anti-HA antibodies. A high
 7 molecular weight smear is observed in cells expressing eFLS2 indicating that the protein is
 8 substituted with HS. No smear or band is evident in lysates from cells transfected with
 9 eS2ED (eS2EDlys) however HSPGs isolated from the conditioned media by anion exchange
 10 (eS2EDcon) contain a high molecular weight smear indicating that the constitutively secreted
 11 syndecan-2 core protein is glycanated. (C) The full length form of syndecan-2 is expressed
 12 on the cell surface. Flow cytometry using the anti-HA antibody was performed on the cell
 13 lines indicated.

14

15

1 Supplemental Figure 4



2

3 **A) Quantification of haemoglobin content of Matrigel plugs.** Plugs containing S2ED
 4 (white bar) contained less blood than GST control (black bar). Error bars represent the
 5 highest and lowest mean measurements obtained from 2 mice injected with 2 plugs.

6 **B) S2ED is inhibitory to angiogenic sprout formation from murine aortas.** Murine aortic
 7 rings were seeded in collagen I gels containing 0.5 μ M of GST or S2ED and supplemented
 8 with 1% FBS and VEGF. (Statistical analysis was performed using a Student's t-test, n=5;
 9 p<0.05).

1 **C) S2ED inhibits migration of ECs from different vascular beds.** Scratch wound
2 migration assays were performed in the presence of 0.5 μ M of the fusion proteins indicated on
3 the EC cell lines from brain, lung and skin. Percentage scratch wound closure was measured
4 after 9 hours of incubation. Proteins containing the adhesion regulatory domain S2ED (white
5 bars) and S2ED Δ L⁷³-G¹²³ (black bars) inhibit EC migration whereas migration is unaffected
6 by treatment with GST (grey bars) and S2ED Δ P¹²⁴-G¹²³ (light grey bars).

7 **D) S2ED slows EC migration through Matrigel.** S2ED inhibits EC invasion through
8 Matrigel. GST or S2ED (0.5 μ M) was incorporated into Matrigel layers in transwells. After 6
9 hours migrated cells were counted and the data represents the mean of triplicate assays.

10 **E) EC microtubule formation is not affected by S2ED.** sEND cells were seeded on layers
11 of Matrigel and micrographs were obtained at the times indicated.