Supplementary Materials for

Actin-propelled Invasive Membrane Protrusions Promote Fusogenic Protein Engagement During Cell-Cell Fusion

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Other Supplementary Materials for this manuscript includes the following:

Materials and Methods

Molecular biology

Full-length *eff-1, sns, duf, αPS2,* and *ed* cDNAs were amplified by RT-PCR from total RNA of *C. elegans* or *Drosophila* embryos and subcloned into the pAc-V5His (for S2R+ cells) or pIZ/V5His (for Sf9 cells) expression vectors (Invitrogen). For non-tagged, FLAG-, or HA-tagged constructs, 3' primers with a stop codon or tag sequences were used for RT-PCR. To make GFP- or mCherry-tagged constructs, *GFP* or *mCherry* cDNA was fused to the C-terminus of cell adhesion molecules (*duf* or *sns*) or actin nucleation promoting factors (*wasp* or *scar*) and subcloned into pAc-V5His. To make chimeras between cell adhesion molecules and actin polymerization regulators, full-length cDNA of *wip*, *wasp* or *scar* was fused to the C-terminus of *duf* or *sns* and subcloned in-frame with the V5 tag into pAc-V5His. All constructs were verified by sequencing analysis.

Cell culture, transfection and RNAi

S2R+ cells were grown in Schneider's *Drosophila* medium (Gibco) containing 10% fetal bovine serum (HI FBS, Gibco), and transfected using Effectene (Qiagen) according to the manufacturer's instructions. Sf9 cells were grown in Insectagro SF9 serum free medium (Mediatech) and transfected using ESCORT (Sigma) according to the manufacturer's instructions. For RNAi knockdown, cells were first incubated with 3mg/ml of dsRNA for 4 days, and transfected with appropriate DNA constructs and 100 ng of the same dsRNA. dsRNAs were synthesized using the T7 *in vitro* transcription kit (MEGAscript, Ambion) with gene-specific primers containing the T7 promoter sequence (Table S1). dsRNAs

were phenol-chloroform extracted and purified using NucAway^{TM} Spin Columns (Ambion). dsRNA against DRaptor (*34*) was used as a negative control. RNAi knockdown levels were monitored by RT-PCR, the primers for which spanned at least one exon-exon junction to avoid amplifying any contaminated genomic DNA. RT-PCR of rp49 (ribosomal protein L32) was used as a loading control.

Immunoprecipitation

Immunoprecipitation (IP) experiments were performed as described (*31*). Antibodies used for IP: mouse anti-V5 (1:500; Invitrogen) and mouse anti-FLAG (1:500; Sigma); for western blot: HRP-conjugated mouse anti-V5 (1:5000; Invitrogen) and mouse anti-FLAG (1:5000; Sigma).

Immunostaining

To visualize actin cytoskeletal rearrangement, S2R+ cells transfected with indicated constructs were fixed in 4% paraformaldehyde in a cytoskeleton stabilization buffer (50 mM KCl, 1.37 M NaCl, 40 mM $NaHCO_3$, 4 mM KH_2PO_4, 110 mM Na_2HPO_4, 20 mM $MgCl_2$, 50 mM PIPES, 20 mM EGTA, and 55 mM Glucose) at 48 hrs post-transfection and stained with the following antibodies and reagent in PBSBT (PBS with 0.1% Triton and 0.2% BSA): mouse anti-V5 (1:1000), mouse anti-FLAG (1:1000), rabbit anti-HA (1:1000; Santa-Cruz), and FITC-conjugated phalloidin (1:250 of 20 mM stock solution in methanol; Invitrogen). Secondary FITC-, Cy5-, or Cy3-conjugated antibodies were used at 1:300 (Jackson Immunoresearch).

Cell-cell fusion assays

S2R+ cell intra-group fusion: S2R+ cells grown on coverslips in 6-well plates were transiently transfected with epitope-tagged expression constructs and incubated for 72 hrs. Cells were then fixed and stained with anti-V5 and CellMask^{TM} Orange plasma membrane stain (Invitrogen), and mounted in Prolong Gold antifade reagent with DAPI (Invitrogen). Fusion indexes were calculated as the percentage of nuclei in multinucleate syncytia that contained ≥3 nuclei vs. the total number of nuclei in transfected cells (V5-positive). Thirty randomly chosen 40x microscopic fields from three independent experiments were quantified. To quantify the distribution of nuclei number in multinucleate syncytia, cells in 60 random 20x microscopic field from three independent experiments were counted.

Sf9 cell fusion: Sf9 cell fusion assay was performed as described above with the exception that transfected cells were identified by mCherry expression.

S2R+ cell inter-group fusion: Two groups of S2R+ cells were transfected independently in a 6-well plate. Cells were incubated for 24 hrs, washed and harvested by trypsinization and centrifugation. Harvested cells were washed, resuspended, mixed with the appropriate group of fusion partners at a 1:1 ratio, and seeded onto coverslips. The mixed cell populations were fixed and stained at 48 hrs post-mixing. Inter-group cell fusion was monitored by GFP and FLAG-Merlin co-expression (fig. S2B"'), GFP diffusion (fig. S2C) or mCherry expression (fig. S7).

Cell adhesion assay

S2R+ cells were seeded onto a 6-well plate at a density of $0.5x10^6$ cells/well and transfected with mCherry-tagged plasmids as indicated (Fig. 3C). At 36 hrs post-transfection, the culture plate was sealed with parafilm and agitated on an orbital shaker at 350 rpm for 1 min. Detached cells were withdrawn, re-plated onto a new 6-well plate, and allowed to settle for 1 hr. Cells remained attached to the old culture plate and cells settled on the new plate were quantified under a phase contrast/fluorescent microscope with a 40x lens. Adhesion index was calculated as the percentage of transfected cells (mCherry-positive) vs. the total number of cells in the attached and detached populations, respectively. Thirty randomly chosen 40x microscopic fields from three independent experiments were quantified.

Time-lapse imaging

S2R+ cells transfected with GFP- or mCherry-tagged proteins in 6-well plates were subjected to time-lapse imaging either 24 hrs post-transfection (for intra-group fusion) or 24 hrs post-mixing (for inter-group fusion), using the Nikon live-cell imaging system on the Nikon Eclipse T*i* inverted microscope with a Stage Top Incubator (INU series) and a 40x long-range lens. Multiple sample points (~10) were monitored using Hoffman modulation contrast (HMC) (or phase contrast) and fluorescence microscopy with the Perfect Focus System for over 20 hrs. Images were processed and converted to TIFF files using the NIS Elements software. Movies were created using NIH Image J and Adobe Photoshop CS4.

Confocal Microscopy

Fixed samples were imaged on an LSM 700 Meta confocal microscope with Fluor 20x, 40x, and 63x Oil DIC objectives and Argon 458, 477, 488, 514 nm, HeNe 543nm, and HeNe 633nm lasers. The pinhole was set to 1.0 AU for each channel. Images were processed using Adobe Photoshop CS4.

FRAP analysis

Fluorescence Recovery After Photobleaching (FRAP) analysis was preformed as previously described (*30*) with the following modifications. Cells were seeded on coverslips and transfected with Eff-1, Sns-mCherry and WASP-GFP (or Scar-GFP). At 36 hrs post-transfection, coverslips with the live cells were mounted on slides with S2R+ cell medium, and were loosely sealed with nail polish for stabilization and sufficient air circulation. Fluorescent Sns-mCherry and WASP-GFP (or Scar-GFP) foci at cell-cell contact sites were visualized by a Zeiss EC Plan-Neofluar 40x 1.3 oil lens on the LSM 700 system. mCherry- and GFP-position foci were photobleached and monitored for fluorescence recovery simultaneously.

STORM analysis

Cells were seeded on coverslips and transfected with indicated constructs (Fig. 2 G and H; Fig. 3 F and G; fig. S12). At 48 hrs post-transfection, cells were fixed and stained with primary antibodies. Secondary antibodies labeled by combinations of photo-switchable activator and reporter dyes were prepared as described (*29*). The Cy3/Alexa 647 activator-reporter pair was used to label the secondary antibody against mouse anti-V5 and the Alexa 405/647 pair was used to label the secondary antibody against rabbit anti-

HA and rabbit anti-Actin (1:500; Sigma). TIRF-based STORM was performed on an N-STORM system (Nikon Instruments Inc.) with Ti-E TIRF, the Perfect Focus System, Apo TIRF 100x 1.49 Oil lens, and 405nm, 561 nm, 647 nm AOTF modulated lasers. The reporter dye was switched to a dark state by bleaching with the 647nm laser at 100% and subsequently activated by exciting the appropriate activator dye using either the 405nm or 561nm lasers. Images were collected with NIS-Elements Ar and analyzed with the NIS-A STORM Analysis software.

Transmission Electron Microscopy (TEM)

To preserve intact cell-cell contact sites, S2R+ cells were fixed directly on the 6-well plate at 48 hrs post-transfection with a solution containing 2.5% glutaraldehyde, 1% sucrose, 0.1M sodium cacodylate and 3mM calcium chloride (pH 7.2-7.4) at 4^{o}C overnight. Subsequently, cells were washed with 0.1M sodium cacodylate buffer containing 3% sucrose and 3mM calcium chloride (pH 7.4), and post-fixed with 0.1M sodium cacodylate buffer containing 1% osmium tetroxide and 0.8% potassium ferrocyanide for 1 hr on ice. The post-fixed cells were stained with 2% uranyl acetate, dehydrated and embedded in EPON (Sigma). Following embedding, small blocks (3 mm diameter) of resin carrying embedded cells were excised from the culture plate and subjected to ultrathin sectioning (70 nm thickness) with an ultramicrotome (Leica; EM UC6). Sections were collected on nickel grids. Lead staining was performed as described (*35*) and images were acquired on a Philips CM120 TEM.

ImmunoEM

To preserve intact cell-cell contact sites, S2R+ cells were fixed directly on the 6-well plate at 48 hrs post-transfection. Note that fixing cells on the culture plate precludes the use of the high-efficiency cryo-immunogold labeling method that requires centrifugation of cells, during which cell-cell junctions are disrupted. Cells were fixed for 15 min at room temperature with a 1:1 mix of 6% paraformaldehyde and medium, followed by overnight fixation at 4^{o}C with 6% paraformaldehyde. The fixed cells were then washed with 0.1M sodium cacodylate buffer and post-fixed with 0.5% osmium tetroxide and 0.4% potassium ferrocyanide in the cacodylate buffer for 1 hr on ice. The post-fixed cells were dehydrated and embedded in EPON. Following embedding, small blocks (3 mm diameter) of resin carrying embedded cells were excised from the culture plate and sectioned with the ultramicrotome. Ultrathin sections (70 nm thickness) were collected on nickel grids. Subsequently, the sections were etched in 5% sodium periodate and 0.5% acetic acid solution for 20 min at room temperature and rinsed with H_2O followed by a Tris buffered solution containing 50 mM NH_4Cl. The etched samples were stained with primary antibodies: rabbit anti-HA (1:20), mouse anti-V5 (1:20), and mouse anti-Actin (1:20; Sigma AC-40), respectively, for 1 hr at room temperature, followed by an incubation with 12 nm gold-conjugated secondary antibodies (1:40; NANOGOLD®) for 2 hrs at room temperature. The samples were then washed with H_2O, post-fixed in 2% glutaraldehyde for 5 min, and stained with 2% filtered aqueous uranyl acetate for 10 minutes. Images were acquired on a Philips CM120 TEM.

Supplementary Figures

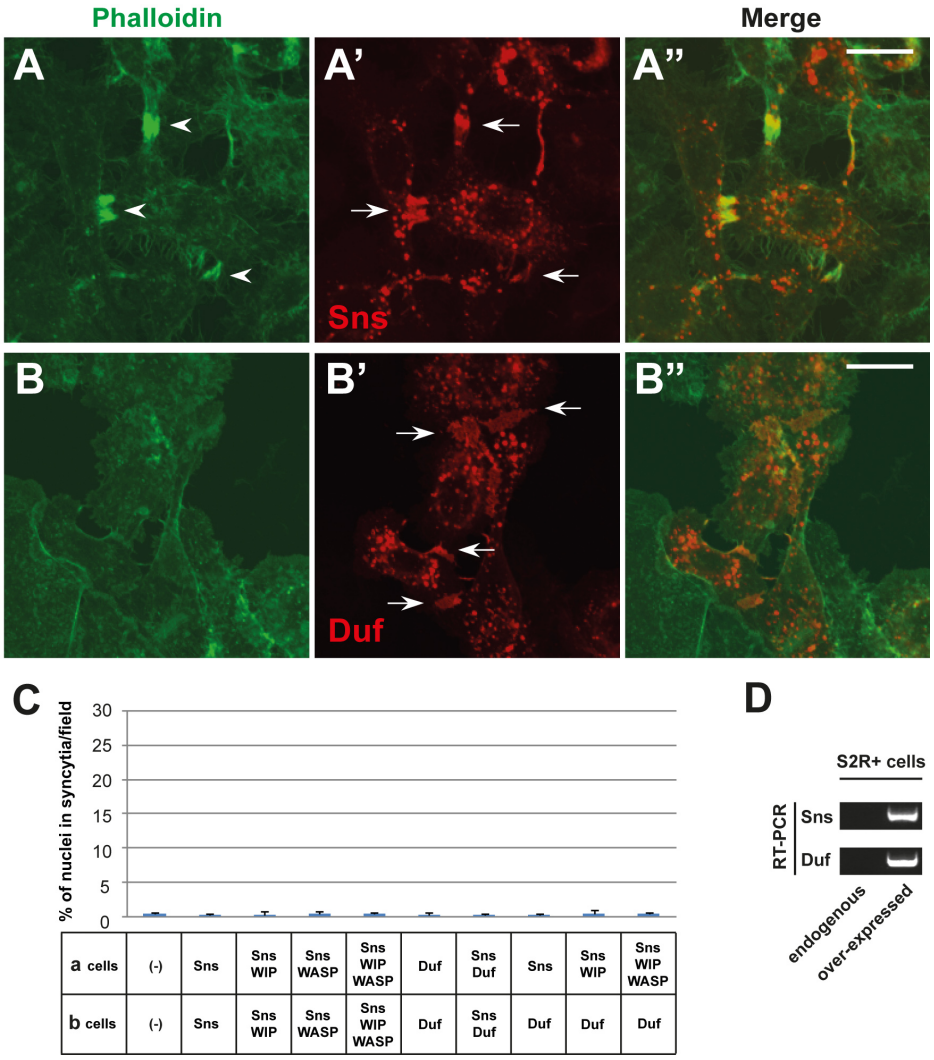

Figure S1. Overexpression of cell adhesion molecules and actin cytoskeletal regulators does not induce cell fusion in S2R+ cells.

Cells transfected with Sns-V5 (A) or Duf-V5 (B) were fixed at 72 hrs post-transfection and stained with phalloidin (green) and anti-V5 (red). F-actin enrichment (arrowheads in A) was observed at cell-cell contact sites marked by Sns accumulation (arrows in A'), but was absent at cell-cell contact sites marked by Duf (arrows in B'). Scale bar: 20 μm (A and B). (C) Two groups of S2R+ cells (a and b) were transfected with indicated plasmids, washed after 24 hrs and mixed at a 1:1 ratio. A tiny percentage (≤ 0.3%) of cells were in syncytia (with ≥ 3 nuclei) at 48 hrs post-mixing, in both untransfected and transfected cells. (D) S2R+ cells do not express Sns and Duf endogenously shown by RT-PCR.

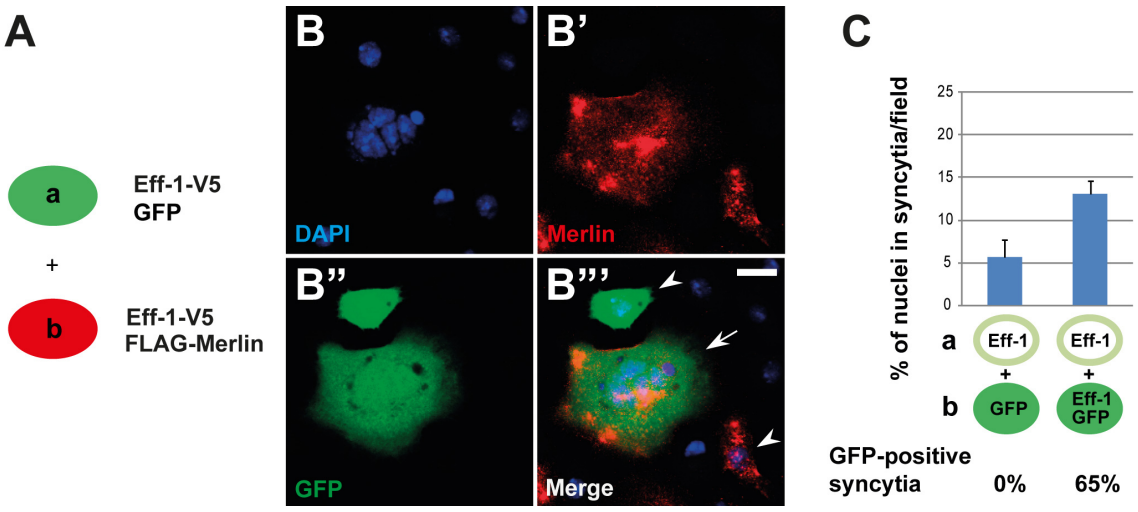

Figure S2. Eff-1 expression in S2R+ cells leads to low frequency cell-cell fusion.

(A to B''') Formation of multinucleate syncytia in Eff-1-expressing cells via cell-cell fusion. Two groups of cells (a and b) were transfected with indicated plasmids (shown in A), washed after 24 hrs and mixed at a 1:1 ratio. Cells were fixed at 48 hrs post-mixing and stained with DAPI (blue; nuclei) and anti-FLAG (red; cytoplasmic FLAG-Merlin) (*36*). The multinucleate syncytium was positive for both GFP and Merlin (arrow in B'''), whereas the mononucleate cells were positive for either GFP or Merlin (arrowheads in B'''). Scale bar: 20 μm. (C) Eff-1 is required in both fusion partners. Two groups of cells (a and b) transfected with indicated plasmids were washed after 24 hrs and mixed at a 1:1 ratio. Note that inter-group fusion, which was indicated by the presence of GFP-positive syncytia, did not occur when only one group of cells expressed Eff-1.

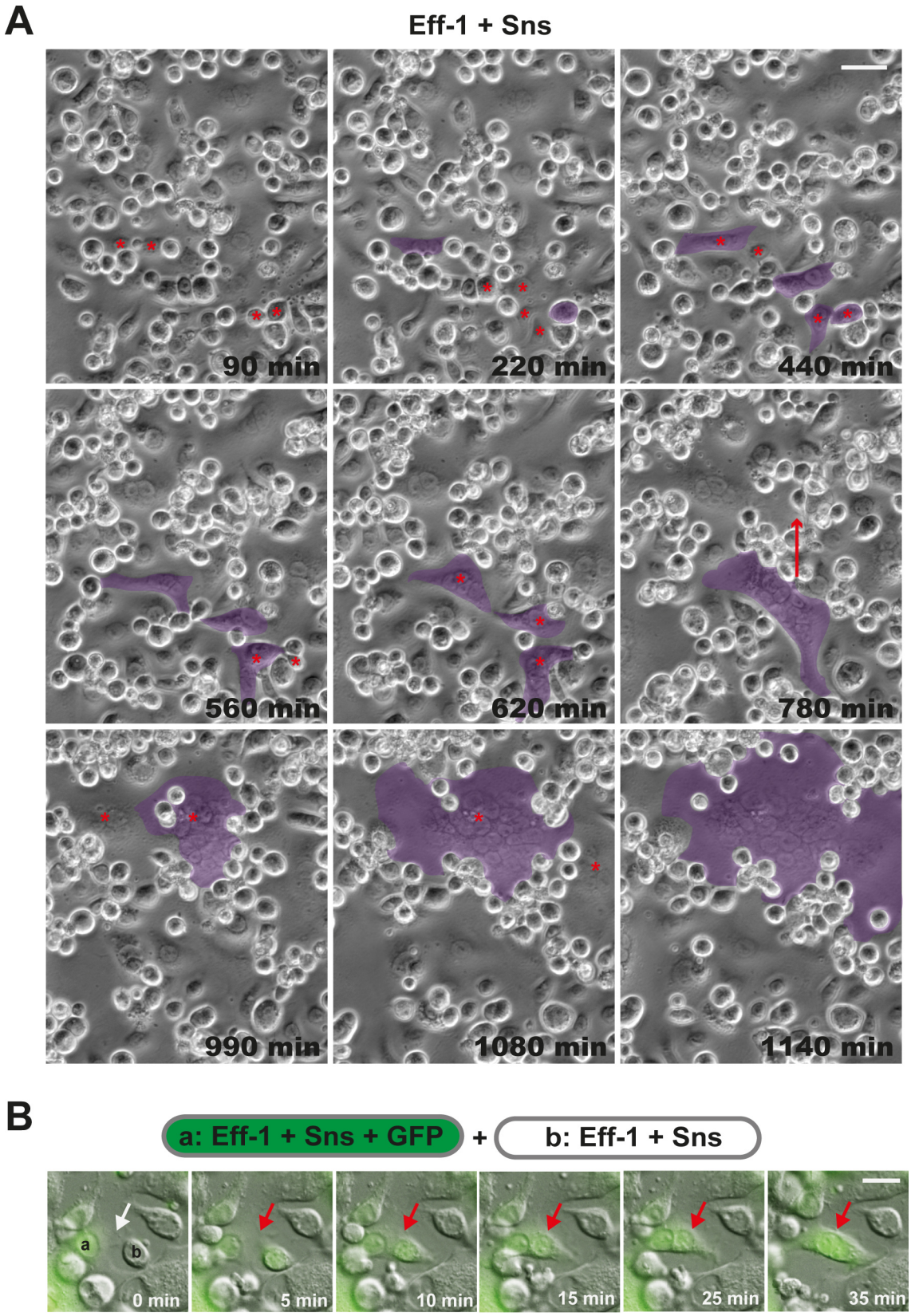
A
Eff-1 + Sns
90 min
220 min
440 min
560 min
620 min
780 min
990 min
1080 min
1140 min
B
a: Eff-1 + Sns + GFP
+
b: Eff-1 + Sns
a
b
0 min
5 min
10 min
15 min
25 min
35 min

Figure S3. Sns-Eff-1-expressing cells form large multinucleate syncytia via cell fusion.

(A) Time-lapse imaging showing step-wise cell-cell fusion events leading to the formation of large multinucleate syncytia. S2R+ cells co-transfected with Sns and Eff-1 were subjected to time-lapse imaging using phase contrast microscopy at 24 hrs post-transfection. Stills from a representative movie (movie S1) are shown. Fusion partners indicated by stars and fused syncytia pseudo-colored purple. Scale bar: 40 μm.

(B) Time-lapse imaging showing GFP diffusion between two fusion partners. Two groups of cells (a and b) transfected with indicated plasmids were washed after 24 hrs, mixed at a 1:1 ratio, and subjected to time-lapse imaging 24 hrs post-mixing. Stills of a representative movie are shown (0 min = 1:39:02 in movie S2). The cytoplasmic GFP diffused from the GFP-positive cell "a" to the GFP-negative cell "b" as a result of cell-cell fusion. White arrow indicates the site of fusion and red arrow indicates the binucleate syncytium. Scale bar: 20 μm.

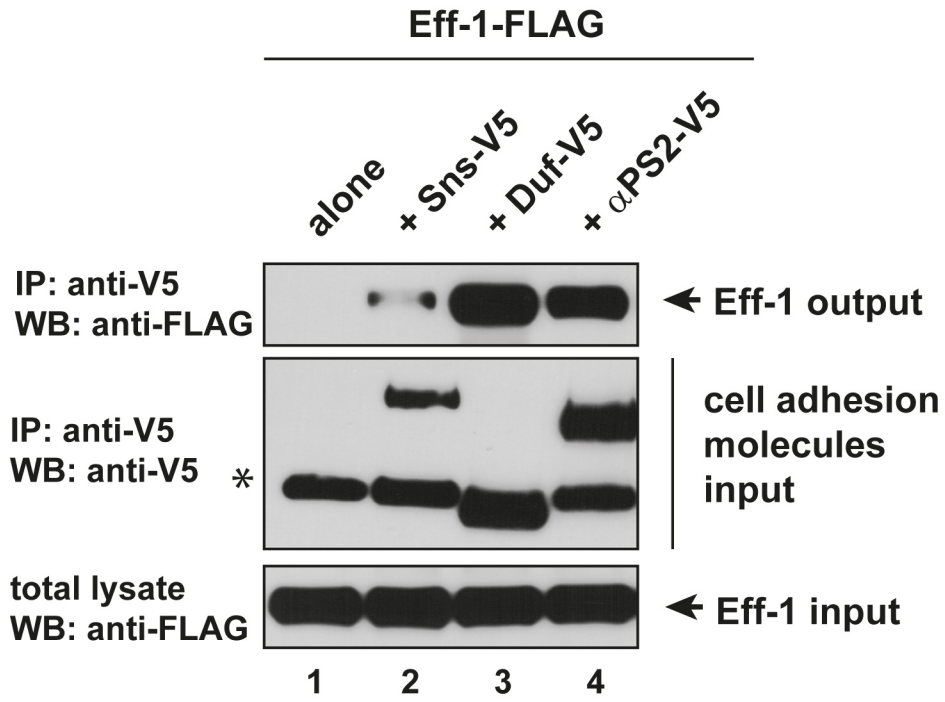

Figure S4. Adhesion molecules bind Eff-1 with different affinities.

Extracts of S2R+ cells expressing indicated proteins (above gel) were immunoprecipitated (IP) and probed (WB) with the indicated antibodies. Note the different affinities between Eff-1 and these adhesion molecules (indicated by Eff-1 output). Asterisk marks a non-specific band present in all lanes, which partially overlapped with the Duf band (lane 3).

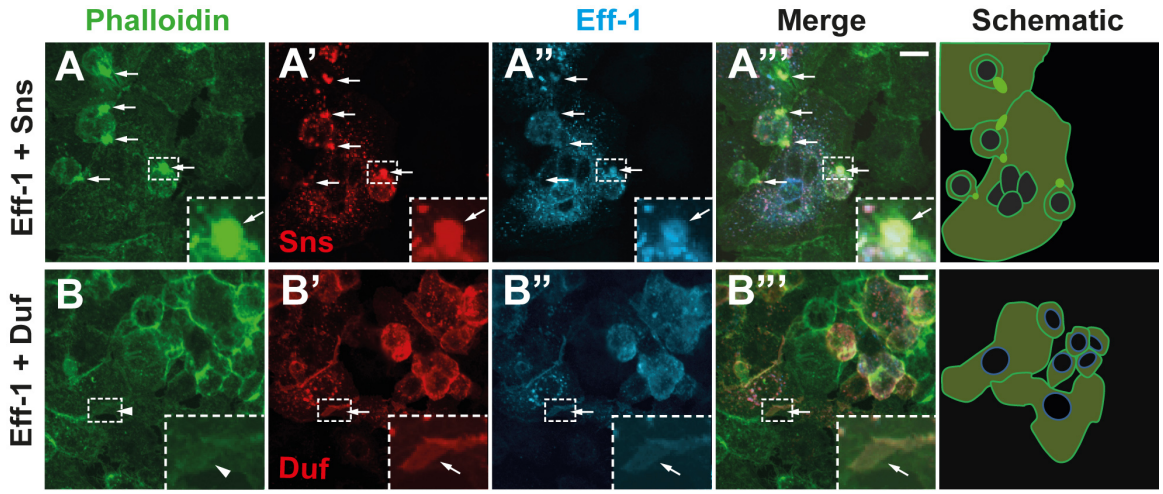

Figure S5. Sns and Duf trigger distinct actin cytoskeletal rearrangements at cell-cell contact sites.

Cells were co-transfected with Sns-V5 (A) or Duf-V5 (B) and Eff-1-HA (A and B), fixed at 48 hrs post-transfection and stained with phalloidin (green), anti-V5 (red) and anti-HA (cyan). Boxed area magnified in the inset and schematic drawing of F-actin shown on the right. Co-expressing Sns with Eff-1 induced the formation of F-actin-enriched foci (arrows in A) associated with Sns and Eff-1 accumulation at cell-cell contact sites (arrows in A' and A"). In contrast, co-expressing Duf with Eff-1 led to Duf and Eff-1 accumulation at cell-cell contact sites (arrows in B' and B"), but without F-actin enrichment (arrowheads in B). Scale bars: 20 μm. See Fig. 1 H and I for GFP-Moesin-labeled F-actin in Sns-Eff-1 and Duf-Eff-1 co-transfected cells.

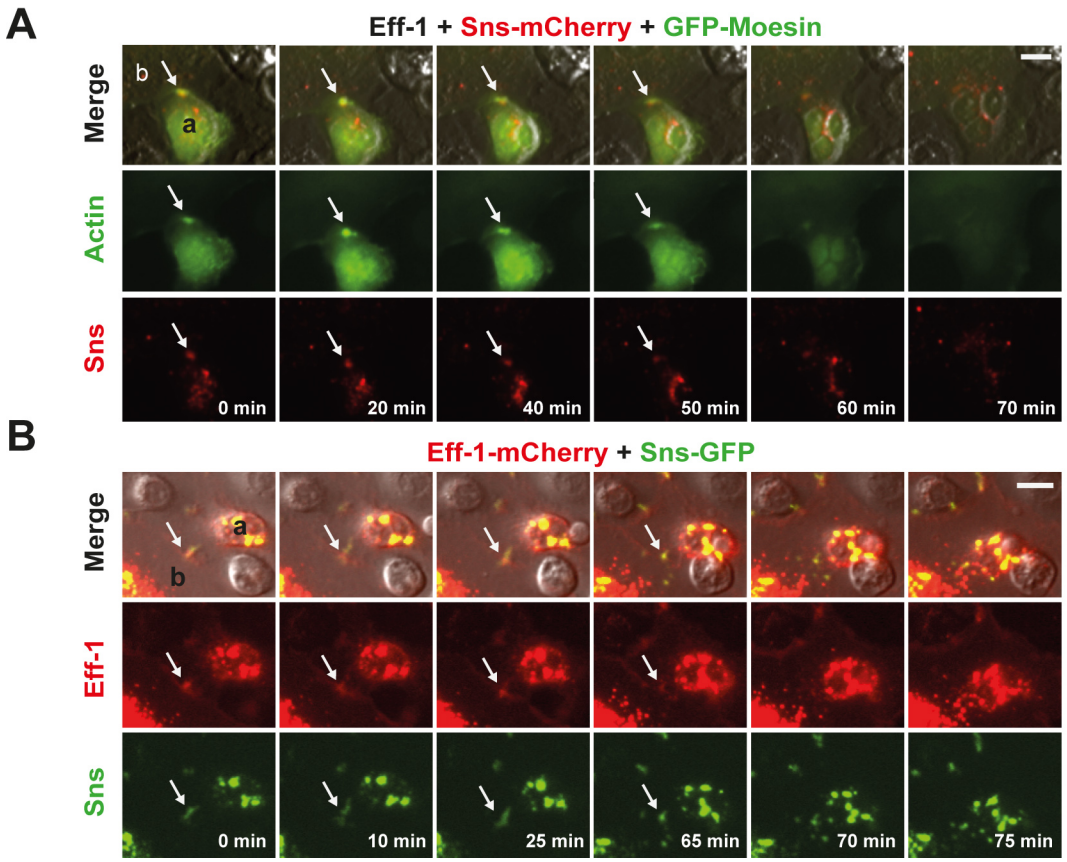

Figure S6. Accumulation of Sns, Eff-1 and F-actin at sites of S2R+ cell fusion.

S2R+ cells transfected with indicated plasmids (above panels) were subjected to time-lapse imaging at 24 hrs post-transfection. Stills from representative movies are shown. (A) Sns-mCherry, GFP-Moesin and merged view with HMC (0 min = 3:00:00 in movie S4). (B) Sns-GFP, Eff-1-mCherry and merged view with HMC (0 min = 00:00:00 in movie S5). Arrows indicate F-actin foci (green in A), Sns (red in A and green in B), and Eff-1 (red in B) at the site of fusion between a pair of fusing cells (a and b).

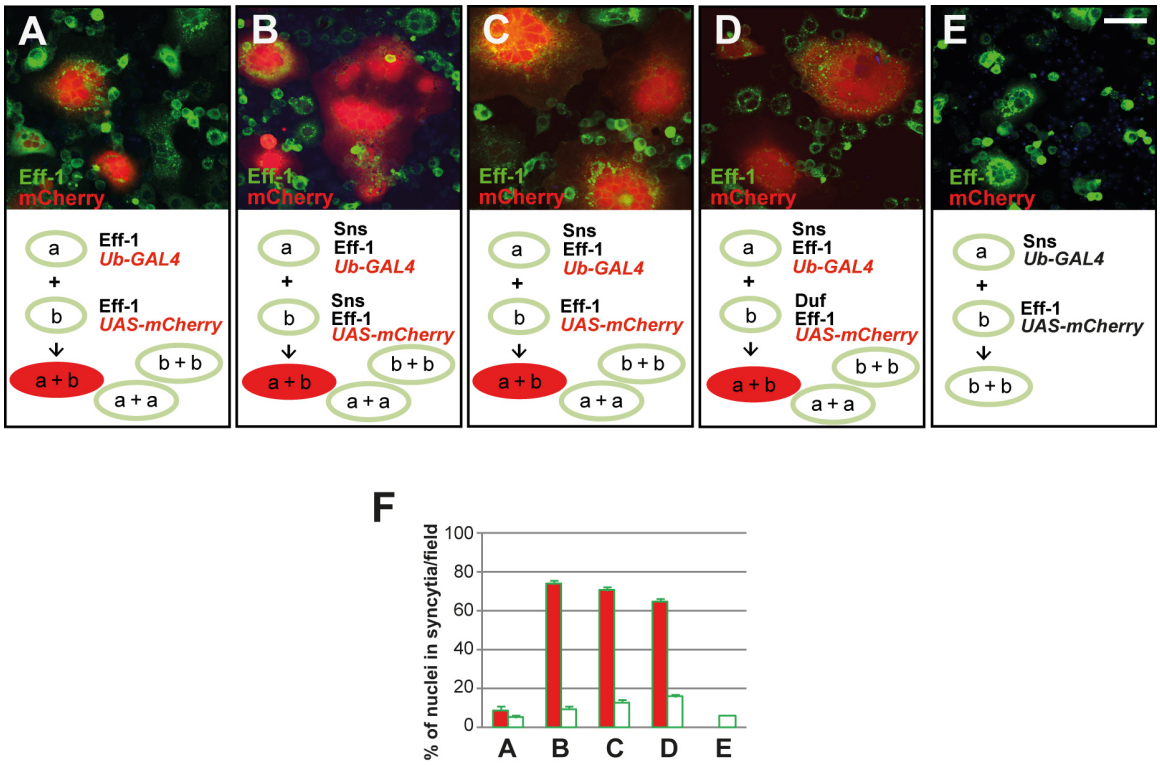

Figure S7. Sns is required in one of the two fusion partners to enhance Eff-1-mediated cell fusion.

(A to E) Inter-group fusion assay of two groups of S2R+ cells (a and b) transfected with indicated plasmids. Ubiquitous (Ub)-GAL4 was expressed in one group of cells and UAS-mCherry in the other, such that inter-group fusion could be indicated by the presence of mCherry-positive multinucleate syncytium. Transfected cells were washed after 24 hrs and mixed at a 1:1 ratio. At 48 hrs post-mixing, cells were fixed and stained with anti-V5 (green; Eff-1-V5), and inter-group fusion was monitored by mCherry expression (red). (A) Eff-1 expression alone in both groups of cells resulted in small red syncytia. (B) Co-expressing Sns-Eff-1 in both groups of cells led to the formation of large red syncytia. (C) Omitting Sns from one group still resulted in the formation of large red syncytia, comparable to (B). (D) Co-expressing Duf-Eff-1 in one group of cells and Sns-Eff-1 in the other resulted in the formation of large red syncytia, comparable to (B) and (C), suggesting that the fusion-enhancing activity of Sns is independent of Duf in S2R+ cells. (E) Omitting Eff-1 from either cell group abolished red syncytial formation. Scale bar: 40 μm. (F) Fusion indexes quantified for experiments in (A) to (E). Red bars and non-filled bars represent mCherry-positive and negative syncytia, respectively.

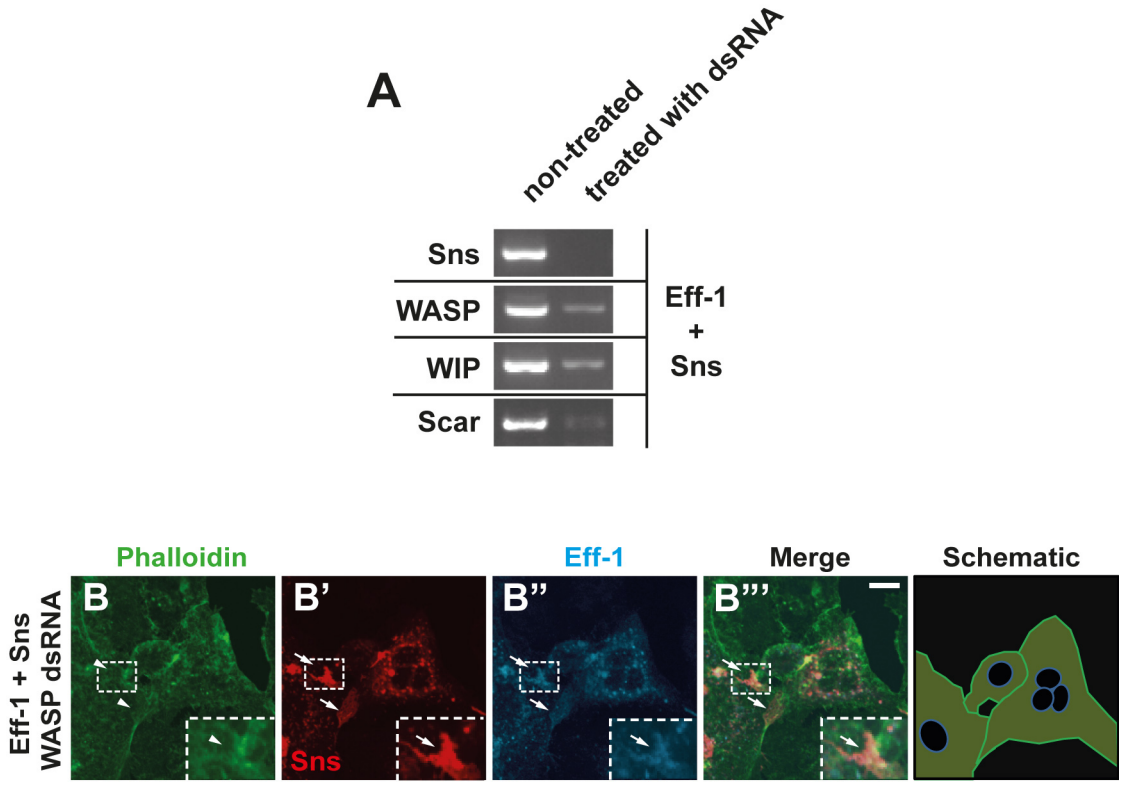

Figure S8. WASP knockdown abolishes F-actin foci formation in Sns-Eff-1-expressing cells.

(A) RNAi knockdown levels of Sns, WASP, WIP and Scar in Sns-Eff-1-expressing cells monitored by RT-PCR. See Fig. 2B for the effects of RNAi knockdown on cell-cell fusion. (B to B"') WASP is required for the formation of F-actin-enriched foci at cell-cell contact sites. Following pre-incubation with dsRNA against WASP for 4 days, cells were transfected with indicated plasmids and dsRNA (left to panel), fixed at 48 hrs post-transfection and stained with phalloidin (green; F-actin), anti-V5 (red; Sns-V5) and anti-HA (cyan; Eff-1-HA). Boxed area magnified in the inset, and schematic drawing of F-actin shown on the right. Note that WASP knockdown abolished the formation of F-actin foci (arrowheads in B) at cell-cell contact sites marked by Sns and Eff-1 enrichment (arrows in B' and B"). Scale bar: 20 μm.

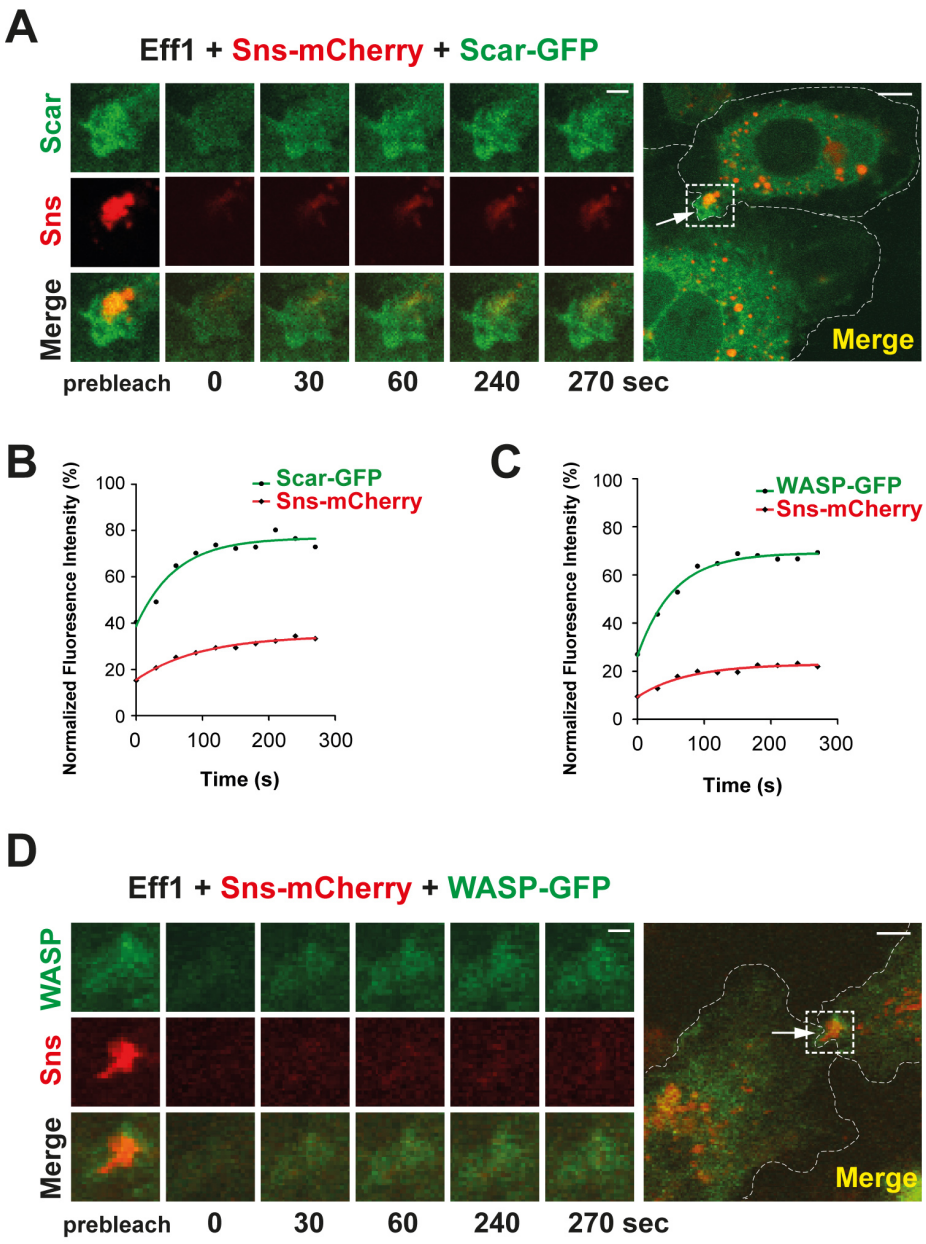

Figure S9. WASP and Scar are more dynamically exchanged than Sns at sites of fusion.

(A and D) S2R+ cells transfected with indicated plasmids (above panels) were subjected to FRAP assay at 36 hrs post-transfection. Note that Scar-GFP (green in A), WASP-GFP (green in D) and Sns-mCherry (red in A and D) all showed enrichment at cell-cell contact sites (arrow in the merge panel). The foci were photobleached and imaged every 30 sec to monitor fluorescence recovery. Sequential images of the boxed area shown on the left. See movie S6 for stills shown in (A) and movie S7 for (D). Fluorescence recovery levels of the foci in (A) and (D) are shown in (B) and (C), respectively. Note the higher level of recovery of Scar and WASP compared with Sns. Scale bars: 5 μm.

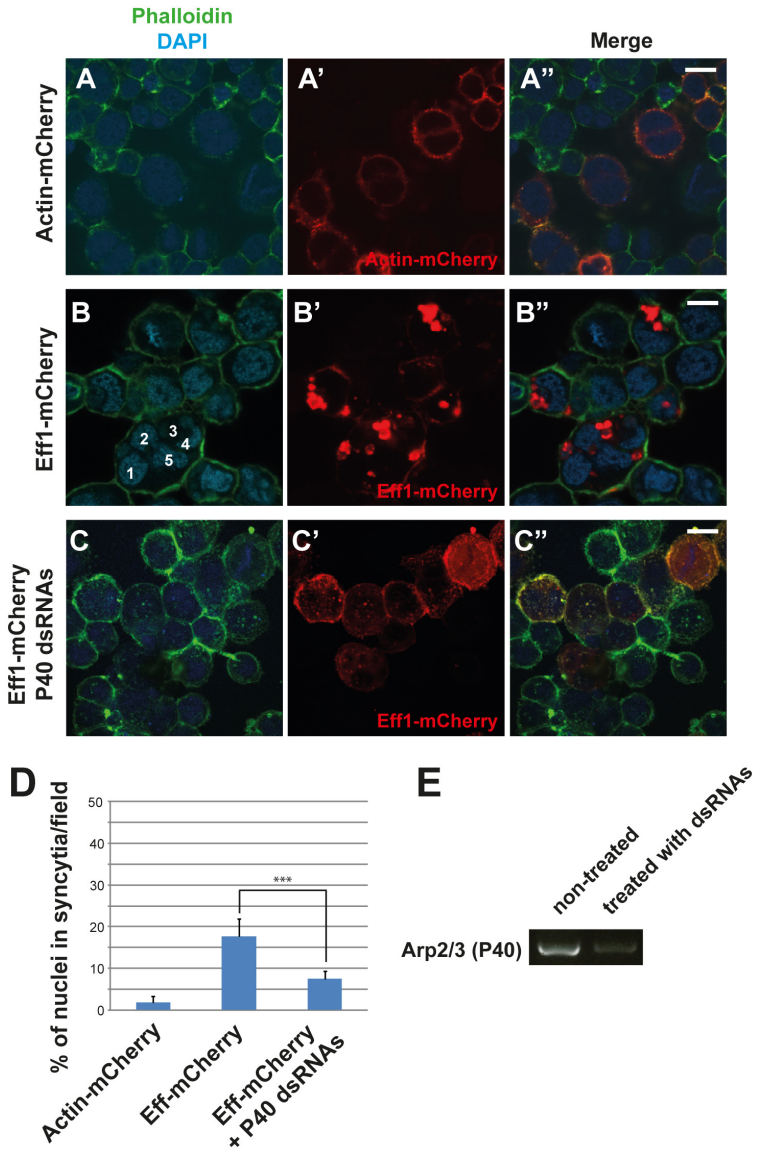

Figure S10. Arp2/3-mediated actin polymerization is required for moth Sf9 cell fusion. (A to C) Cells were transfected with indicated plasmids and dsRNA (left to panel), fixed at 72 hrs post-transfection and stained with phalloidin (green; F-actin) and DAPI (cyan; nuclei). Protein expression was monitored by mCherry (red). The nuclei of a penta-nucleate syncytium are marked (1-5). (C) Cells were pre-incubated with dsRNAs against the P40 subunit of the moth Arp2/3 complex for 4 days prior to transfection. Note that among all the moth Arp2/3 subunits and its NPFs, only P40's DNA sequence is available. Scale bars: 10 μm. (D) Quantification of the fusion index as the percentage of nuclei in multinucleate syncytia (containing ≥3 nuclei) vs. the total number of nuclei in transfected cells. Note that binucleate cells were not included as syncytia in this quantification to avoid any potential confusion with incomplete cytokinesis. Error bars indicate standard deviations. Statistical significance was determined using the two-tailed student's t test ($^{***}p < 0.001$). (E) RNAi knockdown level of P40 in Eff-1-mCherry-expressing Sf9 cells monitored by RT-PCR.

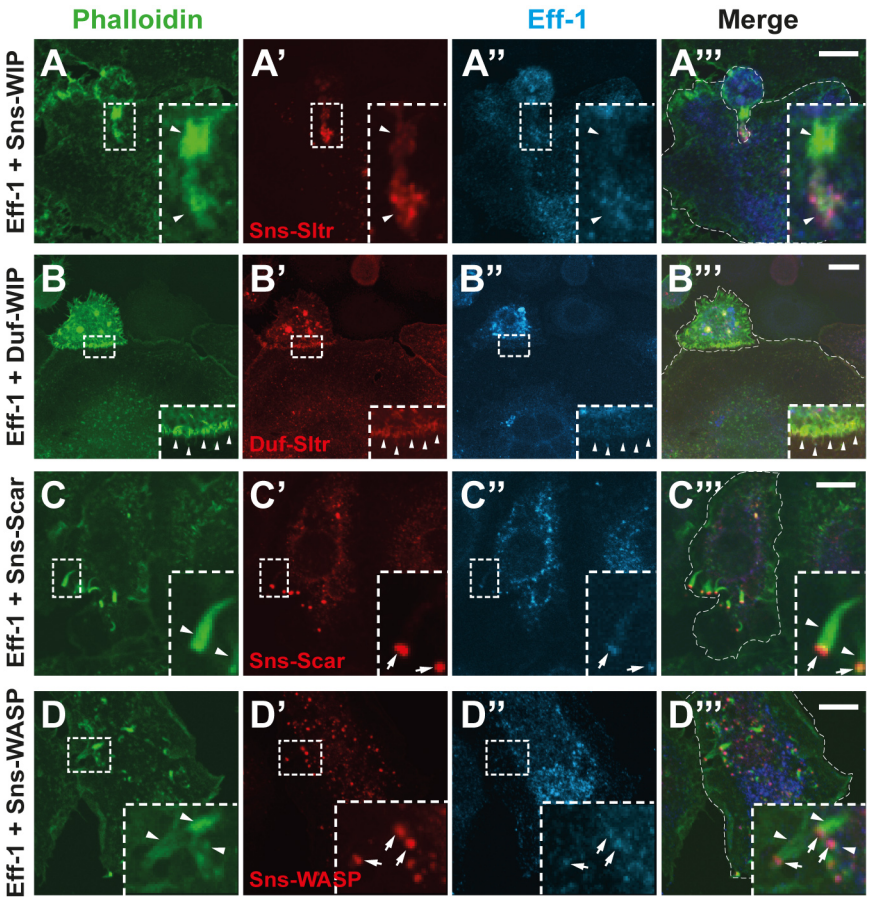

Figure S11. Effects of chimeric proteins of cell adhesion molecules and actin cytoskeletal regulators on actin polymerization.

Cells were transfected with indicated plasmids (left to panel), fixed at 48 hrs post-transfection and stained with phalloidin (green; F-actin), anti-V5 (red; chimeric proteins) and anti-HA (cyan; Eff-1-HA). Boxed area magnified in the inset, cells outlined in the merge panel. (A) Sns-WIP induced F-actin accumulation (arrowheads) at the cell-cell contact site as did Sns (compare with Fig. 1H). (B) Duf-WIP induced F-actin-enriched hair-like protrusions (arrowheads) along the broad cell-cell contact zone. (C and D) Both Sns-Scar (C) and Sns-WASP (D) induced the formation of actin comet tails (arrowheads). These actin comet tails appeared to be propelling vesicles containing the chimeric protein (arrows in C' and D') and Eff-1 (arrows in C" and D") in the cytoplasm (see also movies S8 and S9). Scale bars: 10 μm (A, C, and D); 20 μm (B).

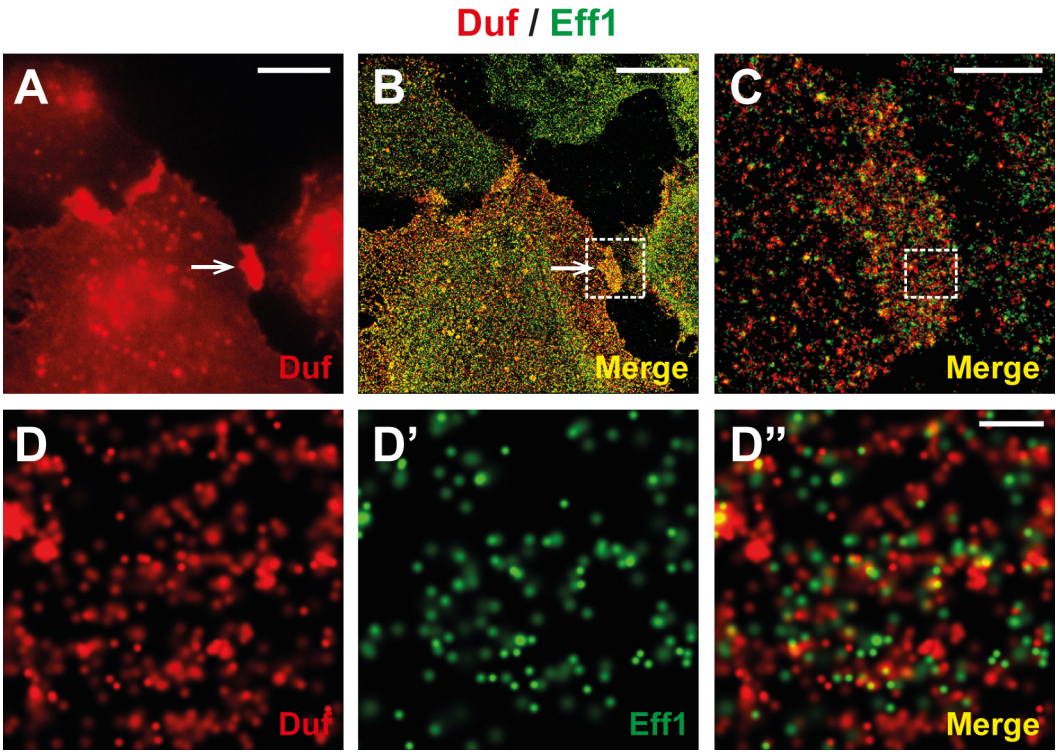

Figure S12. The absence of invasive structures at cell-cell contact sites in Duf-Eff-1-expressing cells.

Cells co-expressing Duf-V5 and Eff-1-HA were fixed at 48 hrs post-transfection, stained with anti-V5 (red) and anti-HA (green), and subjected to STORM analysis. No invasive cellular structures were observed at the cell-cell contact site marked by Duf aggregation (arrow in A and B). Boxed area in (B) enlarged in (C), and that in (C) enlarged in (D to D"). Scale bars: 20 μm (A and B); 500 nm (C); 100 nm (D-D").

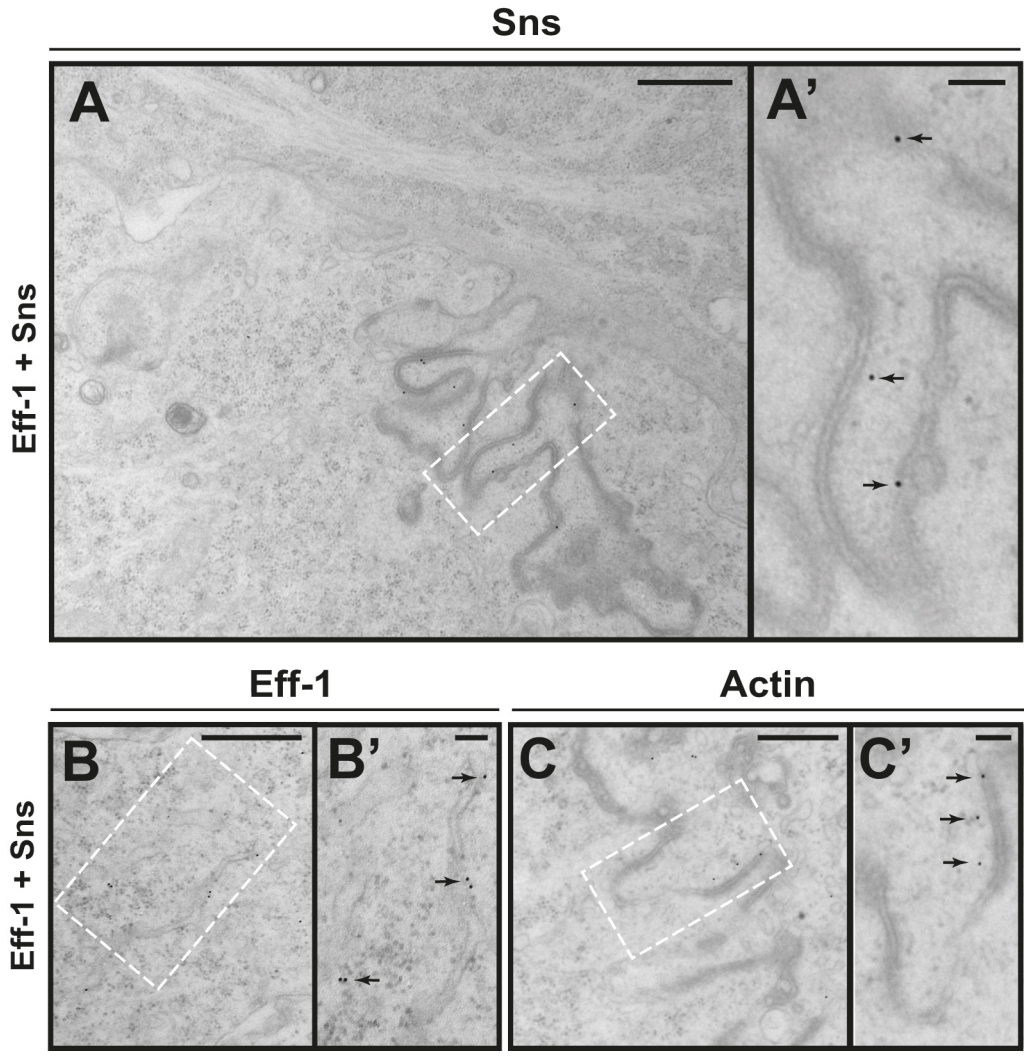

Figure S13. Immunogold labeling reveals Sns and Eff-1 on the plasma membrane along the invasive finger-like protrusions.

Sns-Eff-1-expressing cells were subjected to immunogold labeling (see Materials and Methods) with anti-V5 (Sns-V5) (A-A'), anti-HA (Eff-1-HA) (B-B') and anti-Actin (C-C'). Boxed areas in (A), (B) and (C) enlarged in (A'), (B') and (C'), respectively. (A and A') Immunogold labeling of Sns. Despite the scarcity of signals due to the labeling method (see Materials and Methods), all gold particles (several indicated by arrows in A') were localized at the vicinity of the cell membrane along the invasive fingers, demonstrating high labeling specificity. The slight distance between some gold particles (12 nm in diameter) from the cell membrane (7.5-10 nm in thickness) is likely due to the size of the Ig molecules (20-40 nm in diameter). (B and B') Immunogold labeling of Eff-1. Eff-1 was specifically localized on the membrane along the invasive fingers. (C and C') Immunogold labeling of actin. Although actin is difficult to preserve with the fixation method, gold particles arranged in a tandem pattern were occasionally observed within the invasive fingers (arrows in C'). Scale bars: 500 nm (A, B and C); 100 nm (A', B' and C').

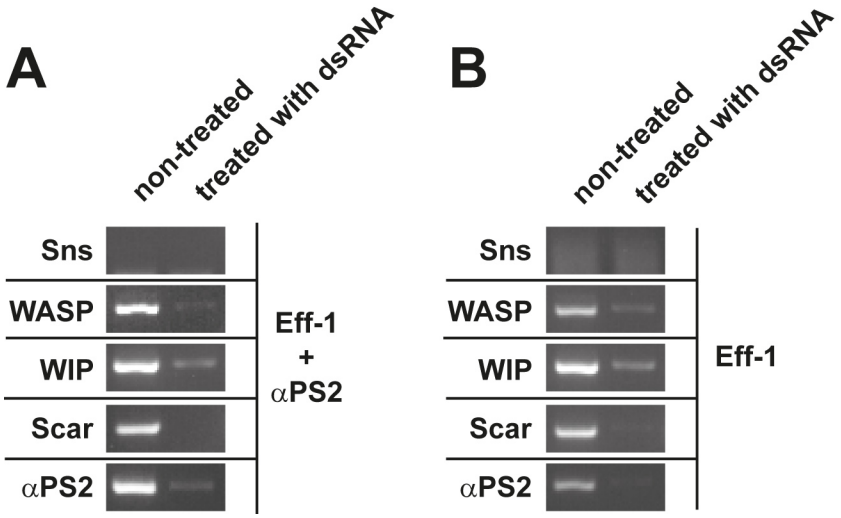

Figure S14. RNAi knockdown of integrin and actin cytoskeletal regulators in S2R+ cells.

RNAi knockdown levels in αPS2-Eff-1- (A) or Eff-1-expressing cells (B) monitored by RT-PCR. The effects of RNAi knockdown on cell-cell fusion are shown in Fig. 3A and B, respectively.

Table S1. PCR primers for making dsRNAs.
* Both PCR primers also contain the T7 promoter sequence (TTAATACGACTCACTATAGGGAGA) at the 5' end.
[#] P40 a and b dsRNAs were used in combination for the knockdown.

Gene name	5'-primer*	3'-primer*	dsRNA length (bp)
wip (fly)	TCTGAGAACAAATCGGGGAC	ACCACCACCAGAACTCGAAC	400
wasp (fly)	CAGCAATTACCCGTTTTCGT	GGAAGCCTTGTGGTCAACAT	504
scar (fly)	CTGGACAGCACAGTTGAGGAG	GAATTCGGTGTGCGGTAGATG	464
sns (fly)	ACGACATGACCGTGAAAACA	GTGACTGTGTCGTGGGTGAC	520
αPS2 (fly)	CGAGATCTTCTACAAGGCGG	ATTATGCACTTGCACCTCCC	428
P40 a (moth)[#]	TGACCGATTTCCCGTTTGCTTTGG	TCACAGTGGAGCGTATTGGCTTCT	489
P40 b (moth)[#]	ATGGGAACAAAGTTGCATGGGTGG	ACTTCAGAACTCGCACGCAAACTG	545

Supplemental movie legends

Movie S1. Time-lapse imaging showing a series of cell-cell fusion events (stills in fig. S3A). S2R+ cells were co-transfected with Eff-1 and Sns, and subjected to time-lapse imaging by phase contrast microscopy. Asterisks indicate cells involved in the stepwise fusion events leading to the formation of a giant multinucleate syncytium. Acquisition time: hrs:min:sec:msec. Scale bar: 15 μm.

Movie S2. Time-lapse imaging showing GFP diffusion between two fusing cells (stills in fig. S3B). S2R+ cells co-expressing Sns-Eff-1 with or without GFP were mixed and subjected to time-lapse imaging. Merged view of fluorescence and HMC microscopy is shown. Note that the cytoplasmic GFP diffused from the GFP-positive cell "a" into the GFP-negative cell "b" resulting from cell-cell fusion. Arrow indicates the site of fusion. Scale bar: 10 μm.

Movie S3. Time-lapse imaging showing a dynamic F-actin-enriched focus at the site of cell fusion (stills in Fig. 2A). S2R+ cells were co-transfected with Eff-1, Sns and GFP-Moesin, and subjected to time-lapse imaging. Note the gradual accumulation of F-actin at the cell-cell contact site and the dynamic shape changes of the F-actin focus prior to fusion between the mononucleate cell "a" and the multinucleate syncytium "b". Scale bar: 10 μm.

Movie S4. Time-lapse imaging showing Sns and F-actin enrichment at the site of fusion (stills in fig. S6A). S2R+ cells co-expressing Eff-1, Sns-mCherry and GFP-Moesin were subjected to time-lapse imaging. Note the association of the green F-actin focus with the red Sns aggregate at the site of fusion (arrow) between syncytia "a" and "b". Scale bar: 10 μm.

Movie S5. Time-lapse imaging showing Eff-1 and Sns aggregates at the site of fusion (stills in fig. S6B). S2R+ cells co-expressing Eff-1-mCherry and Sns-GFP were subjected to time-lapse imaging. Note that Sns and Eff-1 were both enriched at the site of fusion (arrow) between a mononucleate cell "a" and a multinucleate syncytium "b". Scale bar: 10 μm.

Movie S6. FRAP analysis of Scar and Sns foci at the site of fusion (stills in fig. S9A). S2R+ cells co-expressing Eff-1, Scar-GFP and Sns-mCherry were subjected to FRAP assay. The Scar- and Sns-positive foci at the site of fusion (arrow) were photobleached and fluorescence recovery was monitored every 30 sec. Note the higher level of Scar recovery compared with that of Sns. Scale bar: 10 μm.

Movie S7. FRAP analysis of WASP and Sns foci at the site of fusion (stills in fig. S9D). S2R+ cells co-expressing Eff-1, WASP-GFP and Sns-mCherry were subjected to FRAP assay. The WASP- and Sns-positive foci at the site of fusion (arrow) were photobleached and fluorescence recovery was monitored every 30 sec. Note the higher level of WASP recovery compared with that of Sns. Scale bar: 10 μm.

Movie S8. Time-lapse imaging showing actin comet tails induced by the Sns-Scar chimeric protein. S2R+ cells co-expressing Eff-1, Sns-Scar-mCherry and GFP-Moesin were subjected to time-lapse imaging. Note that Sns-Scar-containing vesicles were propelled by actin comet tails to move rapidly through the cytoplasm. See fig. S11C for fixed samples. Scale bar: 10 μm.

Movie S9. Time-lapse imaging showing actin comet tails induced by the Sns-WASP chimeric protein. S2R+ cells co-expressing Eff-1, Sns-WASP-mCherry and GFP-Moesin were subjected to time-lapse imaging. Note that Sns-WASP-containing vesicles, like Sns-Scar-containing vesicles shown in movie S8, were propelled by actin comet tails to move rapidly through the cytoplasm. See fig. S11D for fixed samples. Scale bar: 10 μm.

Movie S10. Time-lapse imaging showing dynamic actin cytoskeletal rearrangement in αPS2-Eff-1-expressing cells. S2R+ cells co-expressing Eff-1, αPS2-mCherry and GFP-Moesin were subjected to time-lapse imaging. Note the dynamic actin polymerization (arrowheads) along the broad cell-cell contact zone prior to fusion between a mononucleate cell "a" and a multinucleate syncytium "b". See Fig. 3D for fixed samples. Scale bar: 10 μm.