Figure S1 (Related to Figure 1)

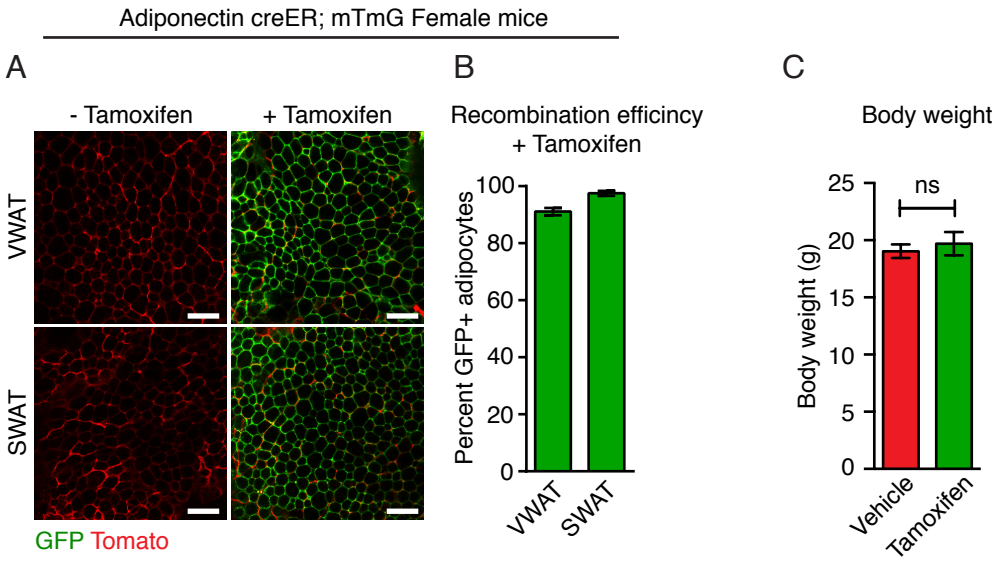

Figure S1 (Related to Figure 1) *Adiponectin-creER* targets nearly all adipocytes in female subcutaneous and visceral depots.

(**A**) Representative confocal images of the indicated depots from *Adiponectin-creER; mTmG* mice before (left) and after (right) tamoxifen treatment. (**B**) Quantification of GFP+ (targeted) adipocytes in the indicated depots following tamoxifen treatment. (n = 3 in each group). (**C**) Body weight measurements from female mice after treatment with tamoxifen or vehicle as performed in the lineage tracing experiments in Figure 1. (n = 5 in each group). Error bars represent mean ± S.E.M. mTmG: membrane-Tomato, membrane-GFP.

Figure S2 (Related to Figure 2)

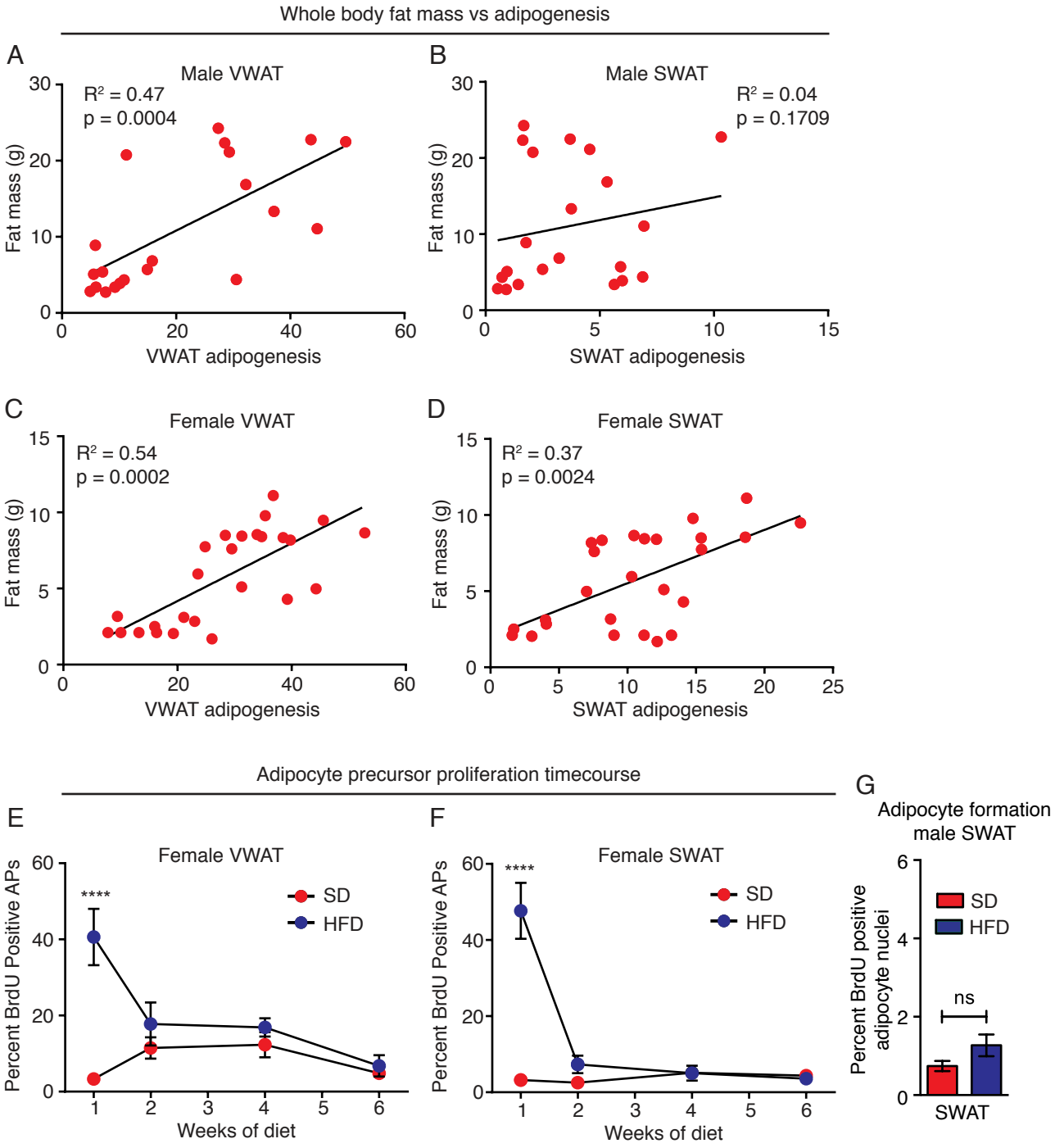

Figure S2. (Related to Figure 2) Adipogenesis in high-fat diet-responsive depots correlates with whole body fat mass.

(**A-D**) Correlation between whole body fat mass and adipogenesis in the indicated depots of male (A-B) or female (C-D) mice. Each point represents one mouse. (n = 21-26 each). (**E-F**) BrdU incorporation into adipocyte precursors at the indicated time points of diet treatment, with BrdU pulse during only the last week before sacrifice. (n = 5 in each group) (**G**) BrdU incorporation into adipocyte nuclei as measured by immunofluorescence in paraffin sections following one week of BrdU treatment (pulse) and 7 more weeks of the indicated diet (chase). (n = 10 in each group). Significance in A-D was determined using Spearman's non-parametric two-tailed correlation analysis. Significance in E-F was determined using a two-way ANOVA with Bonferroni's test for multiple comparisons. Significance in G was determined using an unpaired two-tailed student's t-test. Error bars represent mean ± S.E.M. SD: standard diet, HFD: high-fat diet, AP: adipocyte precursor, BrdU: bromodeoxyuridine.

Figure S3 (Related to Figure 3)

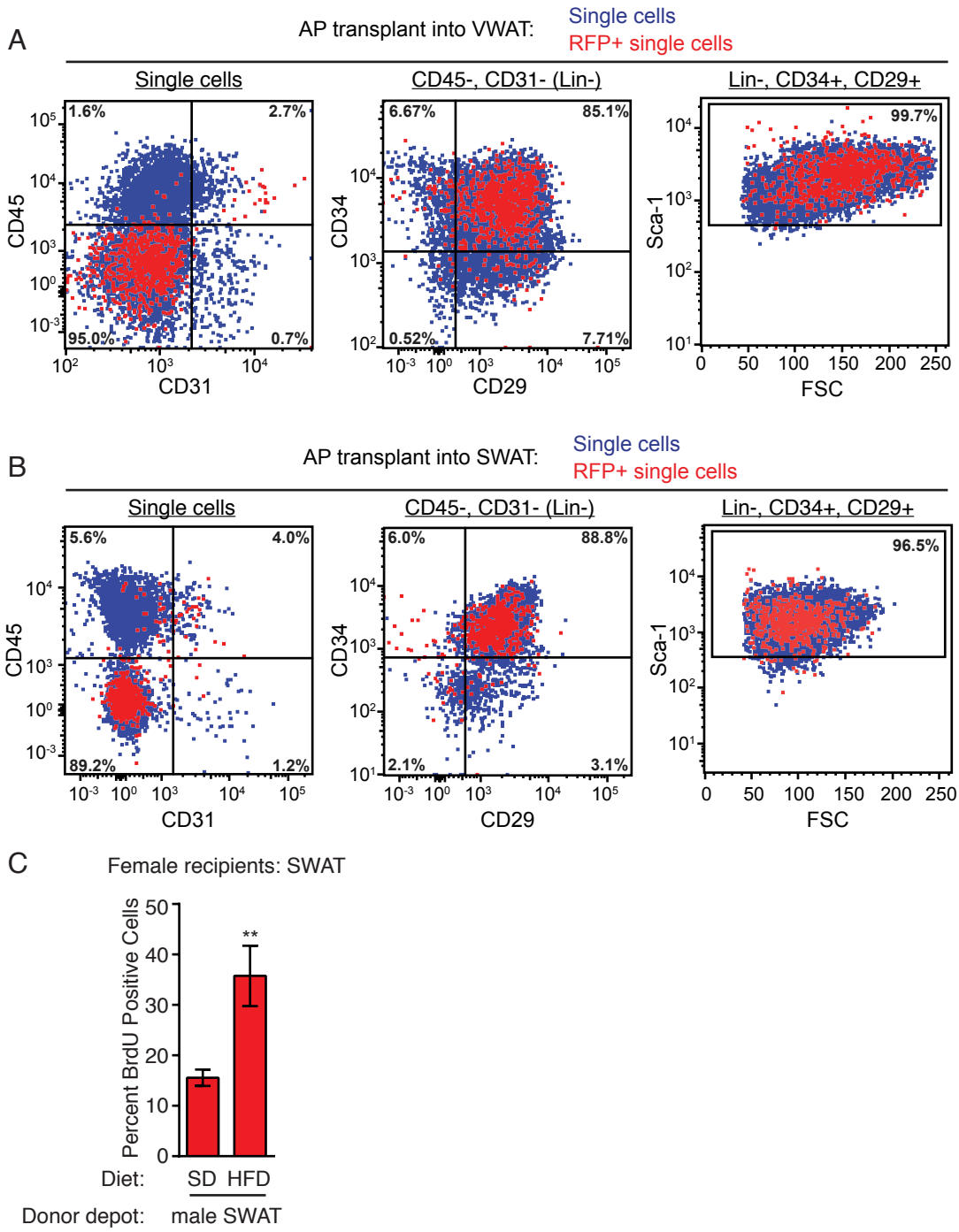

Figure S3. (Related to Figure 3) Characterization of adipocyte precursors after transplantation.

(A-B) Representative flow cytometry plots of cells from VWAT (A) or SWAT (B) in blue, with overlayed plots of transplanted APs in red recovered at the experimental end point. Percentages indicate the percentage of transplanted APs that fall into the indicated gate. Characteristic marker profile of APs is CD45-, CD31-, CD34+, CD29+, Sca1+. (**C**) BrdU incorporation into donor APs derived from male SWAT and transplanted into female SWAT following one week of SD or HFD and BrdU. (SD n = 7, HFD n = 4). **p<0.01. Significance was determined using an unpaired two-tailed student's t-test. Error bars represent mean ± S.E.M. SD: standard diet, HFD: high-fat diet, AP: adipocyte precursor, BrdU: bromodeoxyuridine.

Figure S4 (Related to Figure 4)

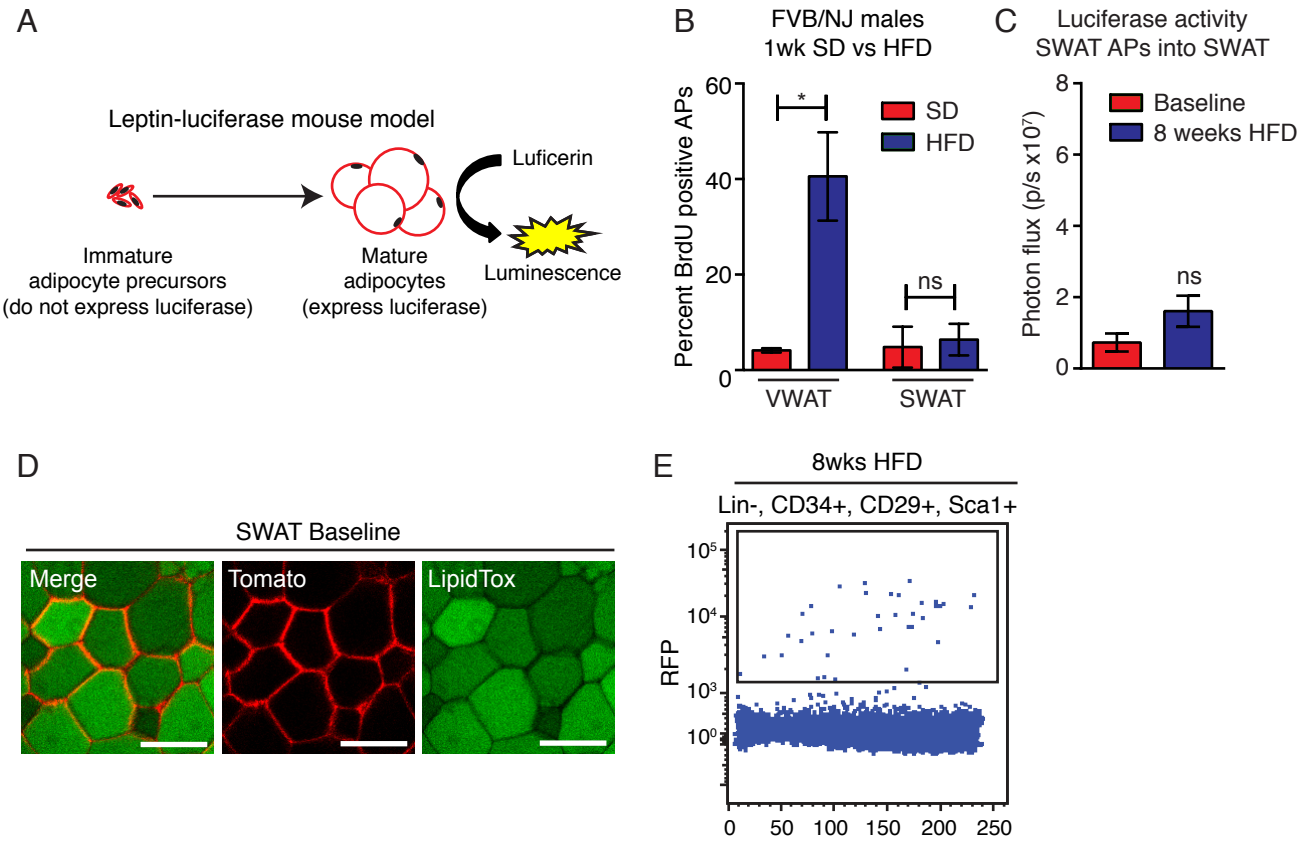

Figure S4. (Related to Figure 4) The male subcutaneous adipose depot does not induce adipogenesis of transplanted adipocyte precursors upon high-fat feeding.

(**A**) Experimental model explaining the mature adipocyte-specific leptin-luciferase mouse model. (B) BrdU incorporation into APs from the indicated depots of adult male FVB/NJ mice following one week of SD or HFD and BrdU. (n = 5 per group) (**C**) Quantification of luminescent signal at the indicated experimental time points following transplant of APs from the SWAT of *leptin-luciferase; mTmG* mice. (n = 5) (**D**) Confocal images of LipidTox+ adipocytes derived from transplanted tdTomato+ APs in SWAT at baseline before starting HFD feeding, demonstrating engraftment of transplanted cells. (**E**) Representative flow cytometry plot of APs from SWAT following transplant and 8 weeks of HFD feeding, demonstrating that tdTomato+ donor cells are still present within the depot. Significance was determined using an unpaired two-tailed student's t-test. Error bars represent mean ± S.E.M. Scale bar is 100μM. HFD: high-fat diet, AP: adipocyte precursor, ns: not significant.

Table S1 (Related to Experimental Procedures)

Figure Panel	Sample sizes
1A-B	Female SD n = 12, HFD n = 15 Male SD n = 12, HFD n = 9
1E	Females SD n = 12, HFD n =15 Males SD n = 6, HFD n = 4
1F	SD n = 6, HFD n = 4
1G	n = 6 per group
2A-B	n = 21
2C-D	n = 26
2F	n = 5 per group
2G	SD n = 8, HFD n = 10
2H	n = 10 per group
2I	SD n = 5, HFD n = 10
3D	SD n = 4, HFD SWAT donor n = 8, HFD VWAT donor n = 3
3E	SD n = 5, HFD SWAT donor n = 5, HFD VWAT donor n = 4
4C	n = 12

Table S1. (Related to Experimental Methods) Experiment sample sizes.
Sample sizes are listed for individual groups in the main figure panels.

Supplemental Experimental Procedures

Animals

All mice used for these studies were on the *C57BL/6J* genetic background, except *leptin-luciferase; tdTomato* mice, and corresponding wild type recipient mice, which are on the *FVB/NJ* background. *Adiponectin-creER* mice were a generous gift from Dr. Evan Rosen (Beth Israel Deaconess Medical Center, Boston, MA) and can now be purchased at Jackson Laboratories (stock #024671). The *mTmG* mice were purchased from Jackson laboratories (stock #007676). Adult wild type mice were purchased from Jackson Laboratories and experiments were performed beginning at 6-8 weeks of age unless otherwise noted. Leptin-luciferase BAC transgenic mice were originally generated on the *C57Bl/6* background (Birsoy et al., 2008) were reproduced by cryoinjection into *FVBJ/N* strain (Rodeheffer et al., 2008). *C57Bl/6 mT/mG* mice were backcrossed into the *FVB/NJ* background for >9 generations, then crossed to leptin-luciferase mice to generate *leptin-luciferase; mTmG (also referred to herein as leptin-luciferase; tdTomato)* mice isogenic with the *FVB/NJ* strain. Ovariectomized female mice were purchased from Jackson Laboratories.

Transplant assay

Isolation of APs was performed as described(Berry and Rodeheffer, 2013) from the indicated depot of male *C57Bl6/J mT/mG* (**Figure 3**) or *FVBJ/N leptin-luciferase; tdTomato* (**Figure 4** and **S2**) mice and pooled from multiple animals to isolate sufficient numbers of APs. Mice were anesthetized with isofluorane and surgeries were performed using sterile technique. 0.5-1 million APs from the indicated depot were re-suspended in 10 µl of sterile PBS and injected into the left visceral or subcutaneous pad of 3-4 week old (**Figure 3**) or 5-6 week old (**Figure 4**) congenic wild type mice. Mice were allowed to recover for 2-3 weeks, then placed on HFD or SD and treated with BrdU for 1 week prior to sacrifice (**Figure 3**) or maintained on HFD for 8 weeks (**Figure 4**). Left and right adipose tissue depots were excised and analyzed separately. Preparation of stromal cells for BrdU analysis was performed as described(Jeffery et al., 2015), and transplanted cells were identified by Tomato fluorescence. For AP proliferation experiments, results were counted only for transplants in which more than 200 individual Tomato-positive donor AP cells were recovered in the recipient depot stromal vascular fraction. For transplant of male cells into male mice, this resulted in a 61% (11/18) success rate for injections of VWAT-derived cells into VWAT, a 67% (4/6) success rate for SWAT-derived cells into VWAT, a 78% (7/9) success rate for SWAT-derived cells into SWAT, and 29% (4/14) success rate for VWAT-derived cells into SWAT. For transplant of male cells into female SWAT, the success rate was 35% (11/31). To visualize luminescence in *leptin-luciferase; tdTomato* transplants, mice were intraperitoneally injected with 100µl of 15 mg/mL luciferin (GoldBio, Cat# LUCK-100), the signal was allowed to develop for 15 min, the mice were anesthetized with isoflurane, and the signal was detected with an IVIS Spectrum imaging system (Caliper Lifesciences).

Confocal microscopy

For *mT/mG* quantification experiments, starting at 8 weeks of age, mice were treated with 50mg/kg tamoxifen (Sigma) dissolved in vegetable oil by intraperitoneal injection for 5 consecutive days, and then allowed to recover for 1 week. Mice were then sacrificed for baseline analysis, or placed on HFD or remained on SD for the 8-week chase period. Inguinal subcutaneous and perigonadal visceral tissue were taken from several regions throughout the depot, and analyzed by whole mount confocal microscopy for tdTomato and eGFP expression. For each data point, at least 500 adipocytes were counted from multiple images from each depot of each animal.

For adipocyte diameter measurements, the area of each adipocyte (in square pixels) was measured using Adobe Photoshop CC. The diameter of each adipocyte was calculated using the measured area, assuming each adipocyte is a perfect circle. At least 300 adipocytes were measured for each data point. Analysis of adipocyte nuclei was performed as described previously (Jeffery et al., 2015). 110-230 adipocyte nuclei were scored for each data point. Images were taken on a Leica TCS SP5 confocal microscope.

Supplemental References

Birsoy, K., Soukas, A., Torrens, J., Ceccarini, G., Montez, J., Maffei, M., Cohen, P., Fayzikhodjaeva, G., Viale, A., Socci, N.D., et al. (2008). Cellular program controlling the recovery of adipose tissue mass: An in vivo imaging approach. Proc Natl Acad Sci U S A *105*, 12985-12990.