# Expanded View Figures

**Figure EV1. Microarray and clustering analysis of Immortomouse™ clonal preadipocyte cell lines.**

A pH measurement of confluent preadipocyte cell lines with different adipogenic differentiation potential. The cells were scored on a scale from 1 to 4 for adipogenic differentiation after Oil Red O staining (1: < 25%; 2: 25–50%; 3: 50–75%, and 4: 75–100% of cells differentiated). Data are shown as mean ± SEM of 4–10 cell lines/group.

B Clonal preadipocyte cell lines used for microarray analysis. Cells were scored on a scale from 1 to 4 for adipogenic differentiation after Oil Red O staining (1: < 25%; 2: 25–50%; 3: 50–75%, and 4: 75–100% of cells differentiated) and 1–4 for media acidification by media color based on scale in Fig 2B (1: pH < 6.5; 2: pH from 6.5 to 6.9; 3: pH from 7.0 to 7.6; 4: pH > 7.7). Cells were selected for differentiation scores of 2 or greater and variable media acidification rates.

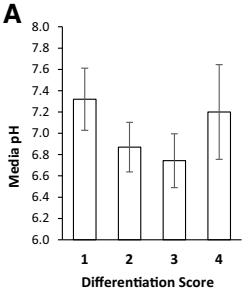
C Affinity propagation clustering of preadipocyte clonal cell line gene expression. Exemplars are representatives of their cluster.

D Hierarchical clustering utilizing the average method of agglomeration of preadipocyte clonal cell line gene expression.

E Principal component analysis of preadipocyte clonal cell line gene expression.

F Bright-field image of clonal preadipocyte lines after *in vitro* adipogenic, osteogenic, and chondrogenic differentiation. Adipocytes, osteocytes, and chondrocytes were stained with Oil Red O, Alizarin Red, and Alcian Blue, respectively. Original photographs were at 10× magnification (left panel). Scale bar = 100 μm.

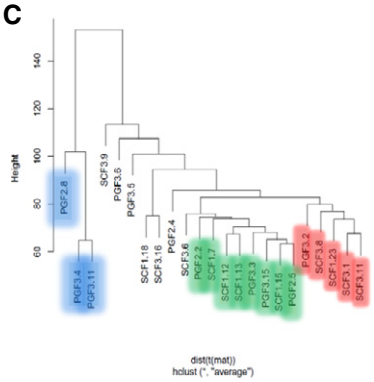
A




B

| Clone | Differentiation Score (1-4) | Media Acidification (1-4) |
|---|---|---|
| PGF 3-2 | 4 | 1 |
| PGF 2-4 | 4 | 1 |
| PGF 3-6 | 2 | 1 |
| PGF 3-3 | 2 | 1 |
| PGF 2-5 | 3 | 3 |
| PGF 3-11 | 2 | 2 |
| PGF 3-15 | 3 | 2 |
| PGF 2-2 | 4 | 3 |
| PGF 3-4 | 2 | 4 |
| PGF 2-8 | 2 | 4 |
| PGF 3-5 | 3 | 4 |
| SCF 1-7 | 3 | 1 |
| SCF 1-18 | 2 | 1 |
| SCF 3-6 | 2 | 1 |
| SCF 1-15 | 2 | 3 |
| SCF 1-17 | 2 | 3 |
| SCF 3-8 | 2 | 2 |
| SCF 1-14 | 3 | 3 |
| SCF 3-9 | 4 | 3 |
| SCF 1-23 | 2 | 4 |
| SCF 3-11 | 2 | 4 |
| SCF 1-12 | 3 | 4 |
| SCF 3-16 | 3 | 4 |
| SCF 3-1 | 4 | 4 |

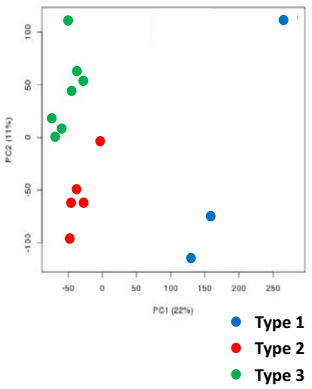
C




D

**Exemplars:**
SCF3.9 SCF3.11 PGF2.5 PGF3.11
**Types:**
Type 1, exemplar SCF3.9:
SCF3.9
Type 2, exemplar SCF3.11:
SCF1.23 SCF3.1 SCF3.6 SCF3.8 SCF3.11 PGF2.4 PGF3.2 PGF3.6
Type 3, exemplar PGF2.5:
SCF1.7 SCF1.12 SCF1.13 SCF1.15 SCF1.18 SCF3.16
PGF2.2 PGF2.5 PGF3.3 PGF3.5 PGF3.15
Type 4, exemplar PGF3.11:
PGF2.8 PGF3.4 PGF3.11

E




F

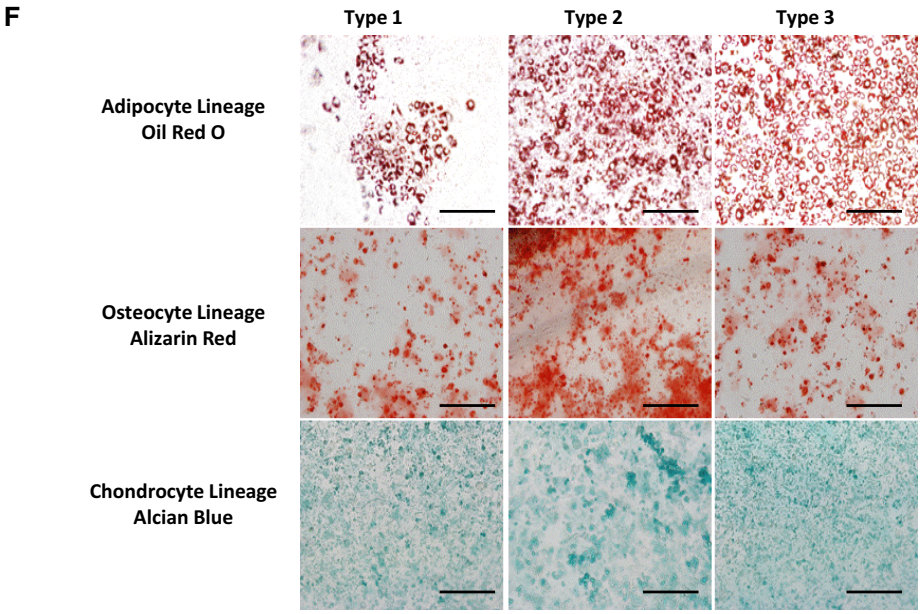



**Figure EV1.**

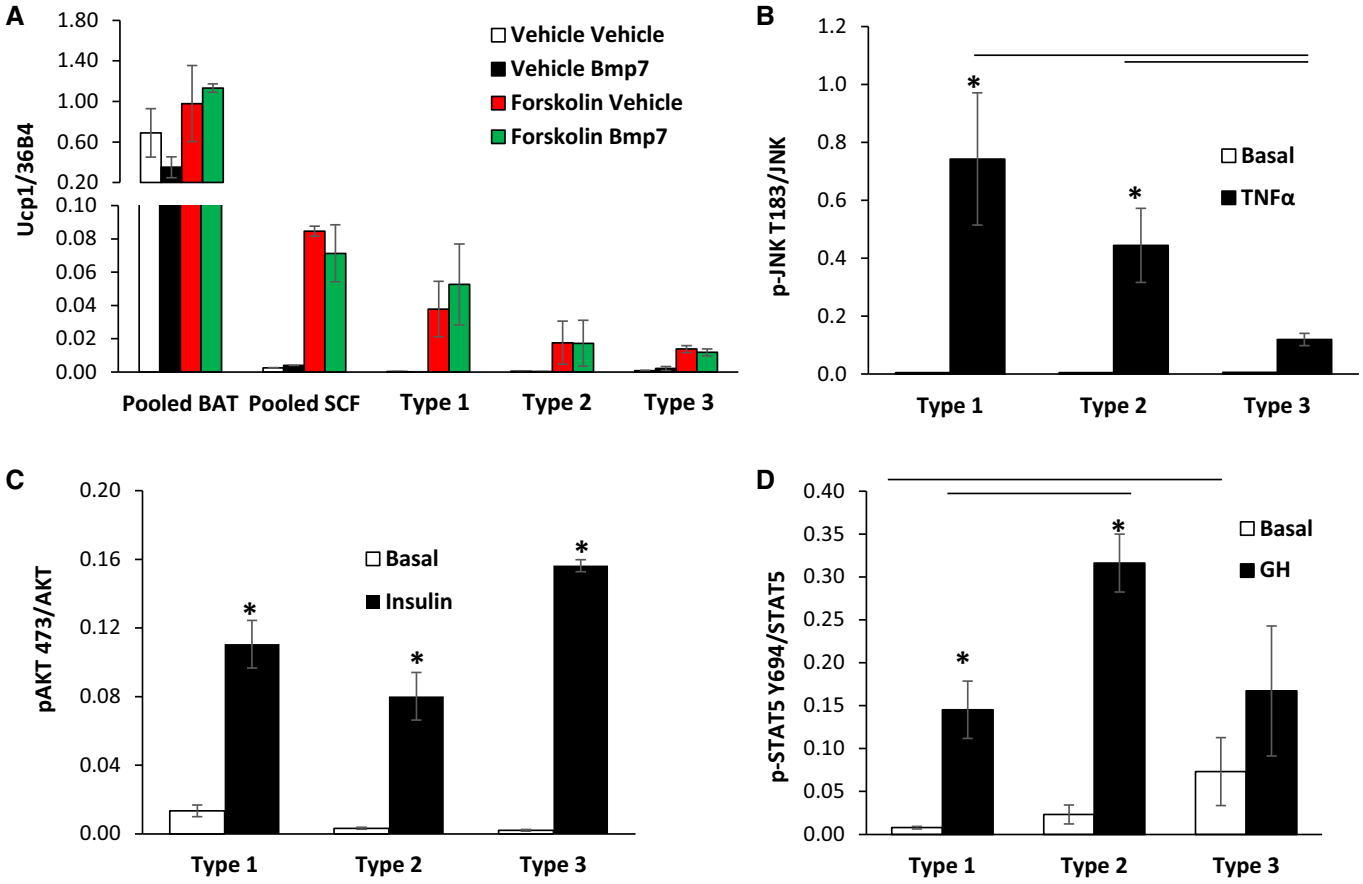



**Figure EV2. Measurement of *Ucp1* expression and quantitation of signaling in preadipocyte cell cultures in response to exogenous stimuli.**

A qPCR analysis for *Ucp1* in RNA isolated from Immortomouse adipocyte cell lines: pooled brown (BAT), pooled subcutaneous (SCF), Type 1, Type 2, and Type 3, after *in vitro* differentiation. Cells were treated ± 3.3 nM BMP7 for 3 days prior to differentiation or treated ± 10 μM forskolin for 4 h. Data are shown as mean ± SEM of 2–7 cell lines per group.

B–D Quantitation of Western blots depicted in Fig 4I. Data are shown as mean ± SEM of three independent cell lines per group; each experiment was performed at least twice. Bars indicate significant differences between groups ($P < 0.05$; two-way ANOVA) in all panels. Asterisks indicate significant differences between basal and treated samples ($P < 0.05$; Student's *t*-test).

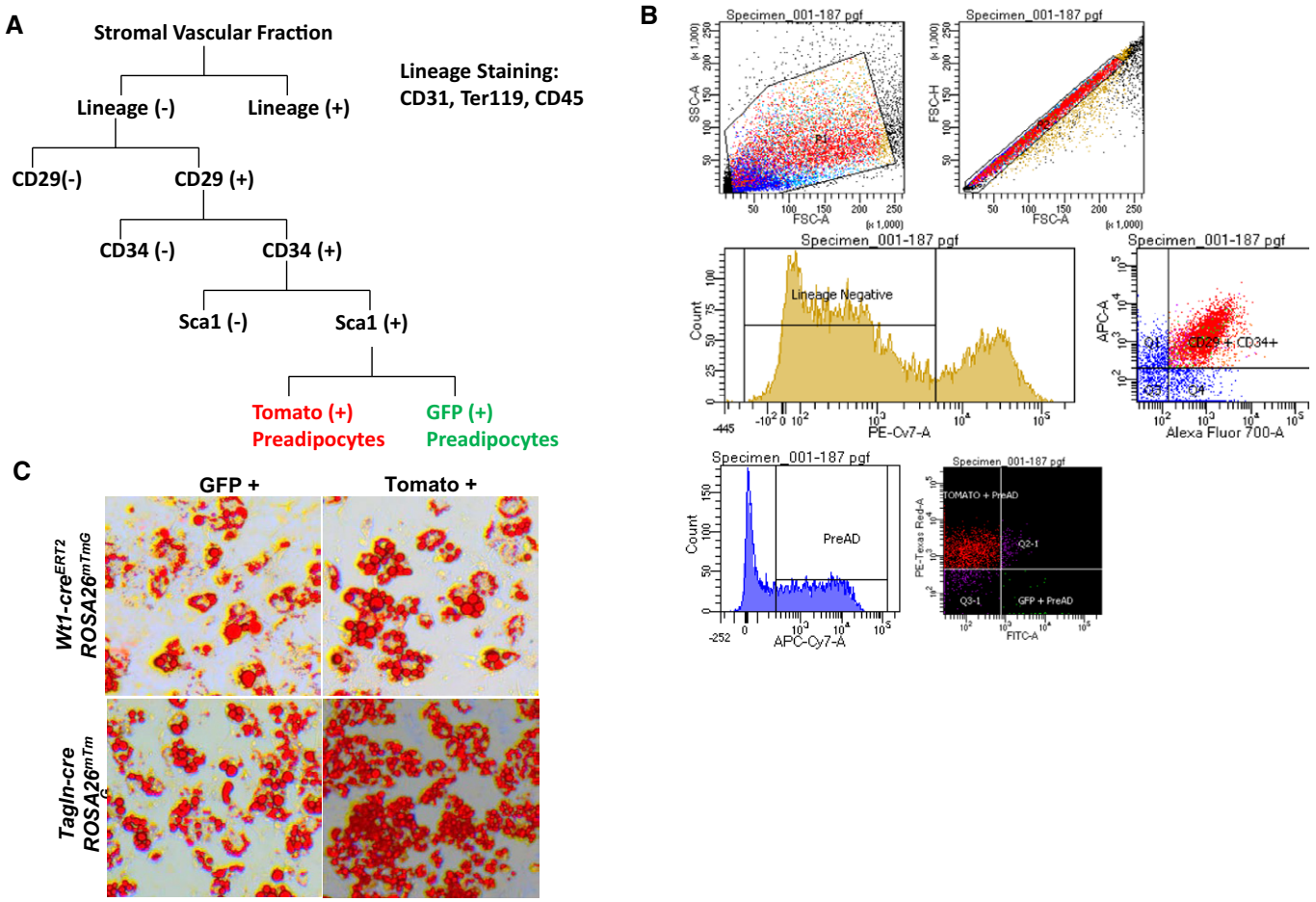



**Figure EV3. FACS Sorting and Adipogenic Differentiation of Rosa26$^{mT/mG}$ preadipocytes.**

A Schematic of FACS sorting for GFP- and Tomato-positive preadipocytes.

B Representative FACS plot of LacZ (+) APCs from the subcutaneous and perigonadal fat depots of Tbx15-LacZ male mice at 6–8 weeks of age.

C Bright-field image of primary mGFP- and mTomato-positive preadipocytes after *in vitro* adipogenic differentiation. Neutral lipids were stained with Oil Red O, and original photographs were at 10× magnification (left panel).

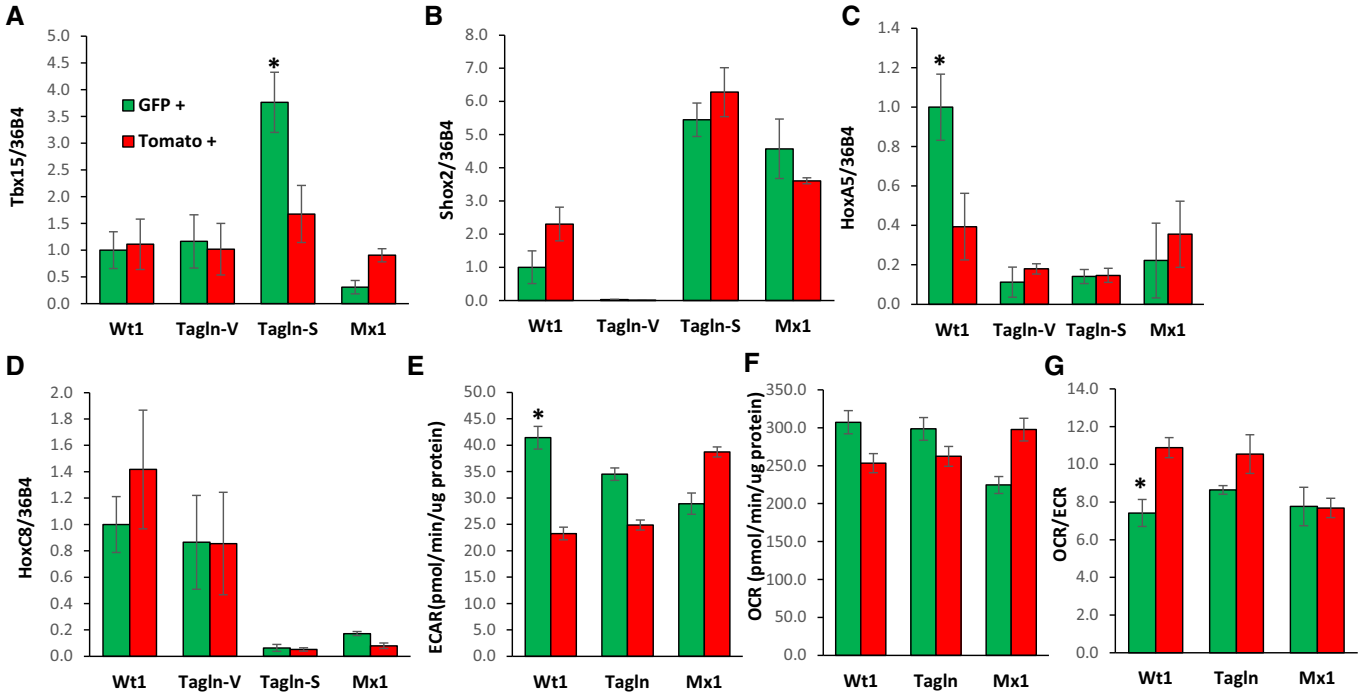



**Figure EV4. Gene expression and maximal metabolic rates of primary preadipocyte subpopulations.**

A–D Expression levels of *Shox2*, *Tbx15*, *HoxC8*, and *HoxC5* mRNA were compared using quantitative real-time PCR (qPCR) in primary preadipocytes isolated from white adipose tissue of 5- to 6-month-old Wt1-cre$^{ERT2}$;Rosa26$^{mT/mG}$, Tagln-cre;Rosa26$^{mT/mG}$, and Mx1-cre;Rosa26$^{mT/mG}$ male mice. mGFP- and mTomato-positive preadipocytes were isolated from the pooled visceral fat depots (perigonadal, perirenal, mesenteric, and pericardial) of Wt1-cre$^{ERT2}$;Rosa26$^{mT/mG}$ mice, the pooled subcutaneous fat depots (subcutaneous and scapular white) of Mx1-cre;Rosa26$^{mT/mG}$, and both the pooled visceral (Tagln–V) and subcutaneous depots (Tagln-S) from Tagln-cre;Rosa26$^{mT/mG}$ mice. Data are shown as mean ± SEM of 4-6 mice/group.

E Maximal extracellular acidification rate (ECAR) of immortalized mGFP- and mTomato-positive preadipocytes was determined by calculating the area under the curve (AUC) during measurements of maximal extracellular acidification rate. The whole experiment was repeated twice. Data are shown as mean ± SEM of three cell lines per group.

F Maximal respiration of preadipocytes was of immortalized mGFP- and mTomato-positive preadipocytes determined by calculating the area under the curve (AUC) during measurements maximal of oxygen consumption rate (OCR). The whole experiment was repeated twice. Data are shown as mean ± SEM of three cell lines per group.

G Ratio of maximal oxygen consumption rate (OCR) to maximal extracellular acidification rate (ECAR) of immortalized mGFP- and mTomato-positive preadipocytes.

Data information: Asterisks indicate significant differences between mGFP- and corresponding mTomato-positive preadipocytes isolated from the same mouse line ($P < 0.05$; paired two-way ANOVA) in all panels. Bars indicate significant differences between mGFP preadipocytes isolated from different mice lines ($P < 0.05$; two-way ANOVA).

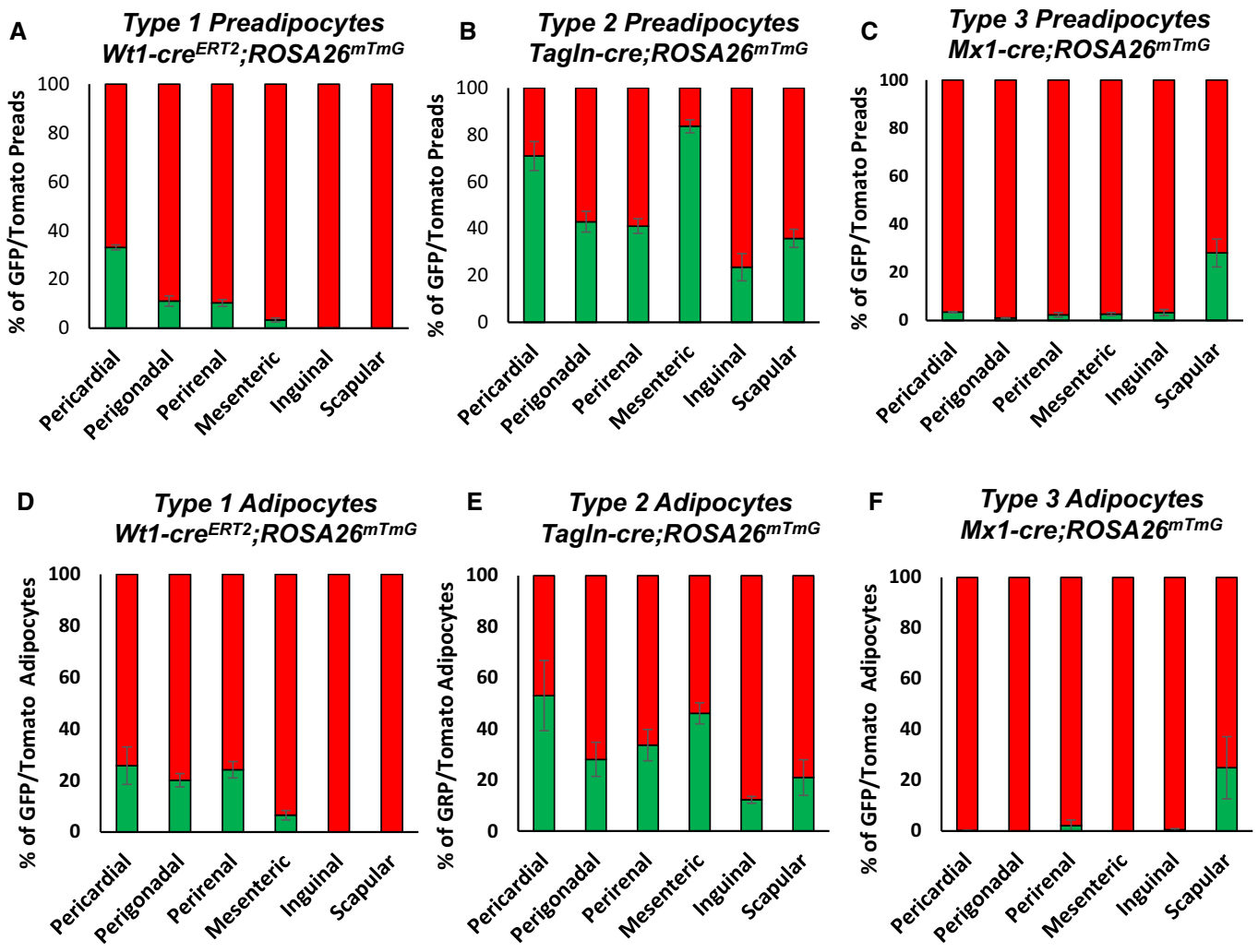



**Figure EV5. Preadipocytes and adipocyte subpopulations in female mice.**

A–C Number of mGFP- and mTomato-positive preadipocytes isolated by FACS from each of the indicated white adipose depots from 5- to 6-month-old female Wt1-cre$^{ERT2}$;Rosa26$^{mT/mG}$, Tagln-cre;Rosa26$^{mT/mG}$, and Mx1-cre;Rosa26$^{mT/mG}$ mice. Data are shown as mean ± SEM of 4–9 mice.

D–F Quantitation of mGFP- and mTomato-positive adipocytes from each of the indicated white adipose depots from 5- to 6-month-old female Wt1-cre$^{ERT2}$;Rosa26$^{mT/mG}$, Tagln-cre;Rosa26$^{mT/mG}$, and Mx1-cre;Rosa26$^{mT/mG}$ mice. Adipocytes were counted from four non-overlapping images/depot/mouse. Data are shown as mean ± SEM of 4–9 mice.