

S1 Genotypes

Fig 1

(A,B) *y w hsFlp/y w;; FRT82 ubiGFP/FRT82 iso, y w hsFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X}*

(C) *y w eyFlp/y w;; FRT82 ubiGFP/FRT82 iso, y w eyFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X}*, (D) *y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 iso, y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 Tsc1^{Q87X}*

Fig 2

(A) *y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 iso, y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 Tsc1^{Q87X}*

Fig 3

(A) *y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 iso, y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 Tsc1^{Q87X}*

(B) *y w eyFlp/y w;; FRT82 ubiGFP/FRT82 iso, y w eyFlp/y w;; FRT82 ubiGFP/FRT82 Rheb^{2G5}, y w eyFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X}, y w eyFlp/y w;; FRT82 ubiGFP/FRT82 Rheb^{2G5} Tsc1^{Q87X}*

(C) *y w hsFlp UAS-GFP/ y w;; tubGal4 FRT82 tubGal80/FRT82 iso, y w hsFlp UAS-GFP/ y w;; tubGal4 FRT82 tubGal80/FRT82 Tsc1^{Q87X}, y w hsFlp UAS-GFP/ y w; UAS-Raptor^{Ri/+}; tubGal4 FRT82 tubGal80/FRT82 iso, y w hsFlp UAS-GFP/ y w; UAS-Raptor^{Ri/+}; tubGal4 FRT82 tubGal80/FRT82 Tsc1^{Q87X}, y w hsFlp UAS-GFP/UAS-S6K^{Ri}; tubGal4 FRT82 tubGal80/ FRT82 iso, y w hsFlp UAS-GFP/UAS-S6K^{Ri}; tubGal4 FRT82 tubGal80/ FRT82 Tsc1^{Q87X}*

Fig 4

(A) *y w hsFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X}, y w hsFlp/y w; FRT40 ubiGFP/FRT40 PTEN¹¹⁷*

(B) *y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 iso, y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 Tsc1^{Q87X}*

(C) *y w hsFlp/y w;; FRT82 ubiGFP/FRT82 iso, y w hsFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X}, y w hsFlp/y w; FRT40 ubiGFP/FRT40 PTEN¹¹⁷*

(D) *y w eyFlp Act>CD2>Gal4/y w;; UAS-lacZ /+, y w eyFlp Act>CD2>Gal4/y w;; UAS-Tsc1^{Ri}/+, y w eyFlp Act>CD2>Gal4/y w; UAS-PTEN^{Ri}/+, y w eyFlp Act>CD2>Gal4/y w; UAS-cherry-dFoxO/+, y w eyFlp Act>CD2>Gal4/y w; UAS-cherry-dFoxO/+; UAS-Tsc1^{Ri}/+, y w eyFlp Act>CD2>Gal4/y w; UAS-cherry-dFoxO/UAS-PTEN^{Ri}*

Fig 5

(A) *y w eyFlp/y w;; FRT82 ubiGFP/FRT82 iso, y w eyFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X}, y w eyFlp/y w; FRT40 ubiGFP/FRT40 PTEN¹¹⁷, y w eyFlp/y w;; FRT82 ubiGFP/FRT82 FoxO²⁵, y w eyFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X} FoxO²⁵, y w eyFlp/y w; FRT40 ubiGFP/FRT40 PTEN¹¹⁷; FRT82 ubiGFP/FRT82 FoxO²⁵*

(B) *y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 iso, y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 FoxO²⁵, y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 Tsc1^{Q87X}, y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 Tsc1^{Q87X} FoxO²⁵*

(C) *y w hsFlp/y w;; FRT82 ubiGFP/FRT82 iso, y w hsFlp/y w;; FRT82 ubiGFP/FRT82 FoxO²⁵, y w hsFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X}, y w hsFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X} FoxO²⁵*

Fig 6

(A) *y w hsFlp/y w;; FRT82 ubiGFP/FRT82 FoxO^{Δ94}, y w hsFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X}, y w hsFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X} FoxO^{Δ94}*

(B) *y w hsFlp UAS-GFP/y w;; tubGal4 FRT82 tubGal80/FRT82 Tsc1^{Q87X}, y w hsFlp UAS-GFP/y w; UAS-p35/+; tubGal4 FRT82 tubGal80/FRT82 Tsc1^{Q87X}, y w hsFlp UAS-GFP/y w;; tubGal4 FRT82 tubGal80/FRT82 Tsc1^{Q87X} FoxO²⁵, y w hsFlp UAS-GFP/y w; UAS-p35/+; tubGal4 FRT82 tubGal80/FRT82 Tsc1^{Q87X} FoxO²⁵*

Fig 7

(A,A') *y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 iso, y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 FoxO²⁵, y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 Tsc1^{Q87X}, y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 Tsc1^{Q87X} FoxO²⁵*

(B) *y w hsFlp/y w;; FRT82 ubiGFP/FRT82 iso, y w hsFlp/y w;; FRT82 ubiGFP/FRT82 FoxO²⁵, y w hsFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X}, y w hsFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X} FoxO²⁵*

Fig 8

(A-D'') *y w eyFlp Act>CD2>Gal4/y w; UAS-FoxO^{Ri/+}; UAS-Tsc1^{Ri/+}*

(E-H) *y w eyFlp Act>CD2>Gal4/y w; UAS-CG33920^{Ri/+}, y w eyFlp Act>CD2>Gal4/y w; UAS-FoxO^{Ri/+}, y w eyFlp Act>CD2>Gal4/y w;; UAS-Tsc1^{Ri/+}, y w eyFlp Act>CD2>Gal4/y w; UAS-FoxO^{Ri/+}; UAS-Tsc1^{Ri/+}, y w eyFlp Act>CD2>Gal4/y w; UAS-PTEN^{Ri/+}, y w eyFlp Act>CD2>Gal4/y w; UAS-PTEN^{Ri}/UAS-FoxO^{Ri}*

S1 Fig

(A,A') *y w eyFlp Act>CD2>Gal4/y w;; UAS-lacZ/+; y w eyFlp Act>CD2>Gal4/y w;; UAS-Tsc2^{Ri}/+*

S2 Fig

(A, A') *y w hsFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X}*

S3 Fig

(A) *y w hsFlp UAS-GFP/y w;; tubGal4 FRT82 tubGal80/FRT82 Tsc1^{Q87X}, y w hsFlp UAS-GFP/y w; UAS-p35/+; tubGal4 FRT82 tubGal80/FRT82 Tsc1^{Q87X}*
(B) *y w hsFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X}*

S4 Fig

(A) *y w hsFlp UAS-GFP/y w;; tubGal4 FRT82 tubGal80/FRT82 iso, y w hsFlp UAS-GFP/y w; UAS-4EBP^{WT}/+; tubGal4 FRT82 tubGal80/FRT82 iso, y w hsFlp UAS-GFP/y w; UAS-4EBP^{AA}/+; tubGal4 FRT82 tubGal80/FRT82 iso , y w hsFlp UAS-GFP/y w;; tubGal4 FRT82 tubGal80/FRT82 Tsc1^{Q87X}, y w hsFlp UAS-GFP/y w; UAS-4EBP^{WT}/+; tubGal4 FRT82 tubGal80/FRT82 Tsc1^{Q87X}, y w hsFlp UAS-GFP/y w; UAS-4EBP^{AA}/+; tubGal4 FRT82 tubGal80/FRT82 Tsc1^{Q87X}*

S5 Fig

(A) *y w hsFlp UAS-GFP/y w; tub-Gal80 FRT40/FRT40 iso; tub-Gal4/EP-Rheb*
(B) *y w eyFlp Act>CD2>Gal4/y w;; EP-Rheb/+*
(C) *y w hsFlp UAS-GFP/y w; tub-Gal80 FRT40/FRT40 iso; tub-Gal4/EP-Rheb*

S7 Fig

(A) *y w hsFlp/y w;; FRT82 ubiGFP/FRT82 iso, y w hsFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X}, y w hsFlp/y w;; FRT82 ubiGFP/FRT82 FoxO²⁵, y w hsFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X} FoxO²⁵*

(B) *y w hsFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X}, y w hsFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X} FoxO²⁵*

S8 Fig

(A,B) *y w eyFlp/y w;; FRT82 ubiGFP/FRT82 iso, y w eyFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X}, y w eyFlp/y w;; FRT82 ubiGFP/FRT82 PKB¹, y w eyFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X} PKB¹, y w eyFlp/y w;; FRT82 ubiGFP/FRT82 FoxO²⁵, y w eyFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X} FoxO²⁵, y w eyFlp/y w;; FRT82 ubiGFP/FRT82 PKB¹ FoxO²⁵, y w eyFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X} PKB¹ FoxO²⁵*

(C) *y w hsFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X}, y w hsFlp/y w;; FRT82 ubiGFP/FRT82 FoxO²⁵, y w hsFlp/y w;; FRT82 ubiGFP/FRT82 Tsc1^{Q87X} FoxO²⁵*

S9 Fig

y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 iso, y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 FoxO²⁵, y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 Tsc1^{Q87X}, y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 Tsc1^{Q87X} FoxO²⁵

S10 Fig

(A) *y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 iso, y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 FoxO²⁵, y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 Tsc1^{Q87X}, y w eyFlp/y w;; FRT82 w⁺*

cl/FRT82 Tsc1^{Q87X} FoxO²⁵

(B) *y w eyFlp/y w;; FRT82 w⁺ cl/FRT82 Tsc1^{Q87X} FoxO²⁵*

(C) *y w eyFlp Act>CD2>Gal4/y w; UAS-FoxO^{Ri/+}; UAS-GFP/UAS-Tsc1^{Ri}*

S1 Video

*y w eyFlp Act>CD2>Gal4/y w; UAS-CG33920^{Ri/+}, y w eyFlp Act>CD2>Gal4/y w;;
UAS-Tsc1^{Ri/+}*

S2 Video

*y w eyFlp Act>CD2>Gal4/y w; UAS-CG33920^{Ri/+}, y w eyFlp Act>CD2>Gal4/y w;
UAS-FoxO^{Ri/+}; UAS-Tsc1^{Ri/+}*

S3 Video

*y w eyFlp Act>CD2>Gal4/y w; UAS-CG33920^{Ri/+}, y w eyFlp Act>CD2>Gal4/y w;
UAS-PTEN^{Ri/+}*

S4 Video

*y w eyFlp Act>CD2>Gal4/y w; UAS-CG33920^{Ri/+}, y w eyFlp Act>CD2>Gal4/y w;
UAS-PTEN^{Ri}/UAS-FoxO^{Ri}*