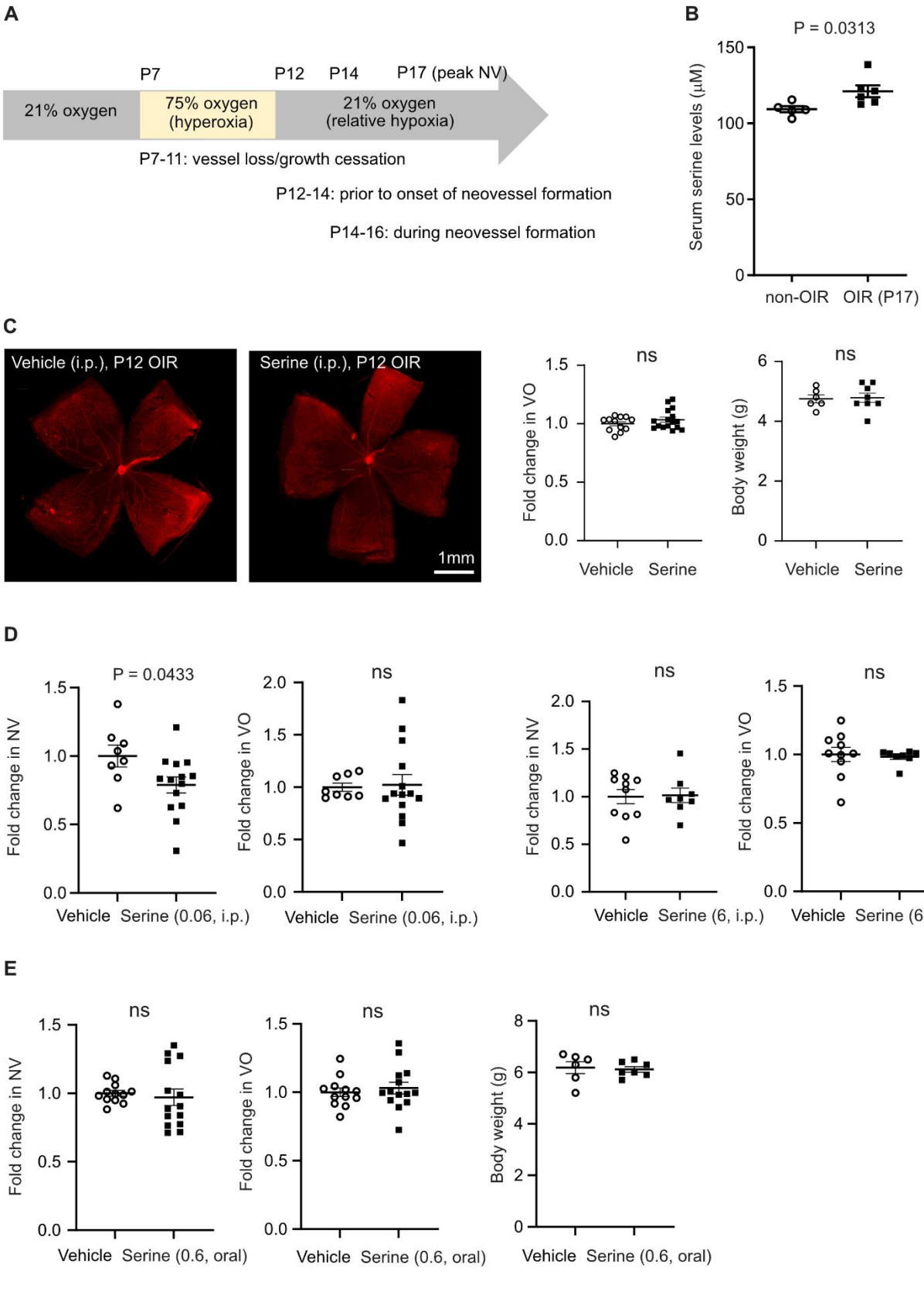
A
P7
P12
P14
P17 (peak NV)
21% oxygen
75% oxygen (hyperoxia)
21% oxygen (relative hypoxia)
P7-11: vessel loss/growth cessation
P12-14: prior to onset of neovessel formation
P14-16: during neovessel formation
B
P = 0.0313
Serum serine levels (μM)
150
100
50
0
non-OIR
OIR (P17)
C
Vehicle (i.p.), P12 OIR
Serine (i.p.), P12 OIR
1mm
ns
Fold change in VO
1.5
1.0
0.5
0.0
Vehicle
Serine
ns
Body weight (g)
6
4
2
0
Vehicle
Serine
D
P = 0.0433
Fold change in NV
1.5
1.0
0.5
0.0
Vehicle
Serine (0.06, i.p.)
ns
Fold change in VO
2.0
1.5
1.0
0.5
0.0
Vehicle
Serine (0.06, i.p.)
ns
Fold change in NV
2.0
1.5
1.0
0.5
0.0
Vehicle
Serine (6, i.p.)
ns
Fold change in VO
1.5
1.0
0.5
0.0
Vehicle
Serine (6, i.p.)
E
ns
Fold change in NV
1.5
1.0
0.5
0.0
Vehicle
Serine (0.6, oral)
ns
Fold change in VO
1.5
1.0
0.5
0.0
Vehicle
Serine (0.6, oral)
ns
Body weight (g)
8
6
4
2
0
Vehicle
Serine (0.6, oral)

Figure S1: Serine treatment did not affect hyperoxia-induced retinal vessel loss.
(**A**) Schematics of mouse OIR model. In mouse OIR, hyperoxia induced retinal vessel loss, followed by relative-hypoxia-induced retinal vessel proliferation (neovascularization, NV).
(**B**) Serum levels of L-serine in P17 OIR vs non-OIR mouse neonates were measured using DL-serine assay kit. n = 5-6 mice per group. Unpaired t-test.
(**C**) Serine (0.6 μg/g) or vehicle was administered by i.p. injection into OIR mice from P7 to P11. At P12, retinal vaso-obliteration (VO, central area without red fluorescence) was examined. n = 12-16 retinas per group. Scale bar, 1 mm. Ratio of change was calculated referring to the average value of littermate vehicle controls. Normality (histogram, QQ-plot, Sapiro-Wilk test) and variance (F-test) were confirmed. Unpaired t-test was used to compare the groups. ns, not significant.
(**D**) Serine (0.06 μg/g, or 6 ug/g) or vehicle was delivered by i.p. injection into OIR mice from P12 to P16. At P17, retinal neovascularization (NV, highlighted in white) and VO was examined. n = 8-14 retinas per group (0.06 μg/g, i.p.), n = 8-10 retinas per group (6 μg/g, i.p.), Scale bar, 1 mm. Fold change was calculated referring to the average value of littermate vehicle controls. No difference in body weight was found (0.06 ug/g: vehicle, 5.3 ± 0.1 g, serine 5.3 ± 0.1 g; 6 ug/g: vehicle, 6.5 ± 0.2 g, serine 6.7 ± 0.1 g). Normality (histogram, QQ-plot, Sapiro-Wilk test) and variance (F-test) were confirmed. Unpaired t-test, or Welch's test, or Mann-Whitney test were used to compare the groups. ns, not significant.
(**E**) Serine (0.6 μg/g) or vehicle was orally delivered to OIR mice from P12 to P16. At P17, retinal NV and VO were examined. n = 12-14 retinas per group. Scale bar, 1 mm. Fold change was calculated referring to the average value of littermate vehicle controls. Normality (histogram, QQ-plot, Sapiro-Wilk test) and variance (F-test) were confirmed. Unpaired t-test, or Welch's test was used to compare the groups. ns, not significant.

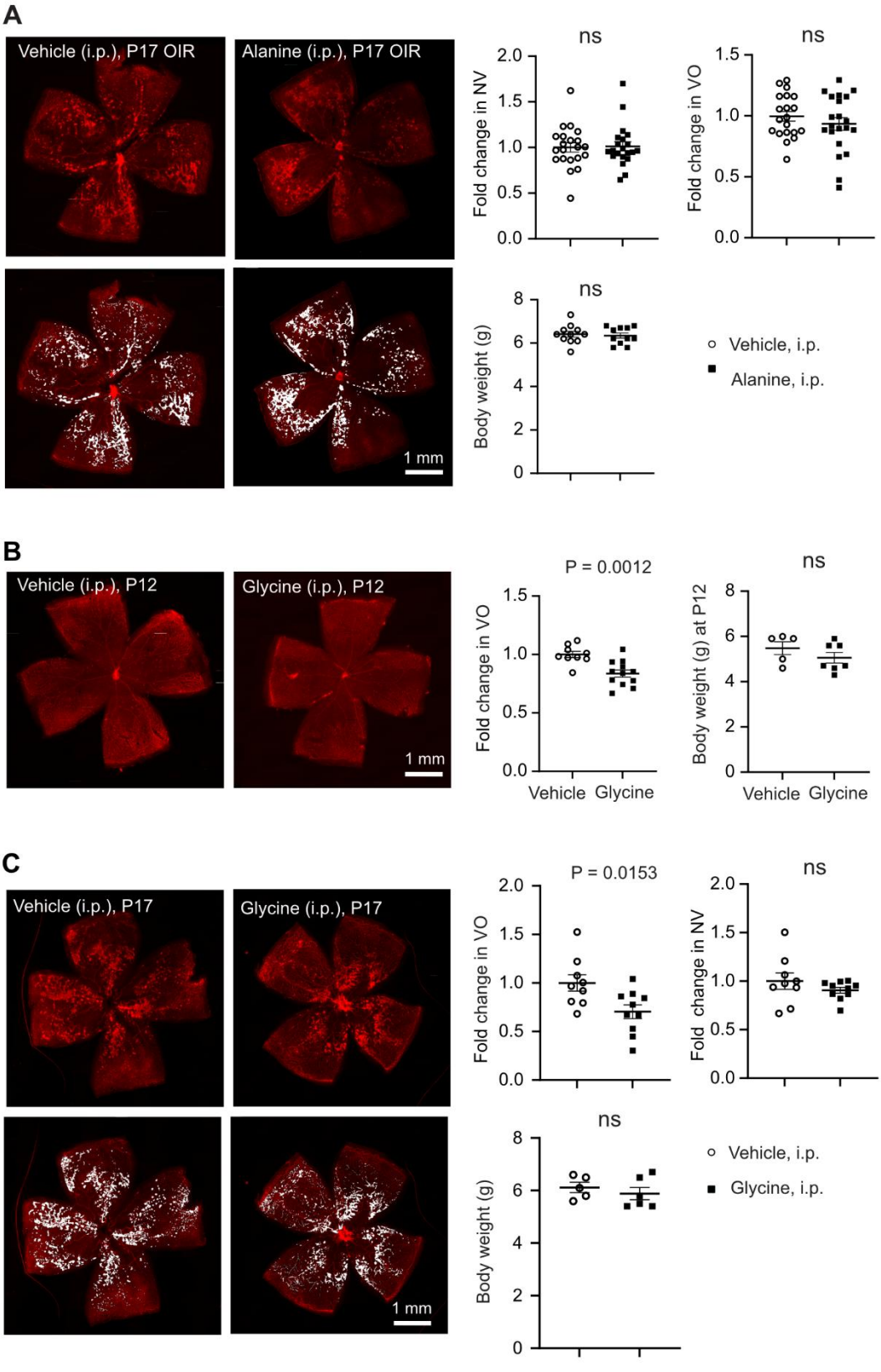
A
Vehicle (i.p.), P17 OIR
Alanine (i.p.), P17 OIR
1 mm
ns
Fold change in NV
2.0
1.5
1.0
0.5
0.0
ns
Fold change in VO
1.5
1.0
0.5
0.0
ns
Body weight (g)
8
6
4
2
0
Vehicle, i.p.
Alanine, i.p.
B
Vehicle (i.p.), P12
Glycine (i.p.), P12
1 mm
P = 0.0012
Fold change in VO
1.5
1.0
0.5
0.0
Vehicle
Glycine
ns
Body weight (g) at P12
8
6
4
2
0
Vehicle
Glycine
C
Vehicle (i.p.), P17
Glycine (i.p.), P17
1 mm
P = 0.0153
Fold change in VO
2.0
1.5
1.0
0.5
0.0
ns
Fold change in NV
2.0
1.5
1.0
0.5
0.0
ns
Body weight (g)
8
6
4
2
0
Vehicle, i.p.
Glycine, i.p.

Figure S2: Glycine treatment promoted retinal re-vascularization (reflected by decreased vaso-obliteration) in OIR mice.

(**A**) Alanine (2 μg/g) was delivered by i.p. injection into OIR mice from P12 to P16. Littermates were treated with vehicle. At P17, retinal vaso-obliteration (VO, central area without red fluorescence) and neovascularization (NV, mid-peripheral area with high intensity of red fluorescence, highlighted in white) were examined. n = 20-21 retinas per group. Scale bar, 1 mm. Fold change was calculated referring to the average value of littermate vehicle controls. Normality (histogram, QQ-plot, Sapiro-Wilk test) and variance (F-test) were confirmed. Unpaired t-test or nonparametric Mann-Whitney test was used to compare the groups. ns, not significant.

(**B-C**) Glycine (0.8 μg/g) was delivered by i.p. injection into OIR mice from P7 to P11 (**B**) or P12 to P16 (**C**). Littermates were treated with vehicle. At P12, P17, retinal vasculature was examined. n = 9-10 retinas per group. Scale bar, 1 mm. Unpaired t test. Fold change was calculated and compared with littermate vehicle controls. Normality (histogram, QQ-plot, Sapiro-Wilk test) and variance (F-test) were confirmed. Unpaired t-test or Welch's t-test was used to compare the groups. ns, not significant.

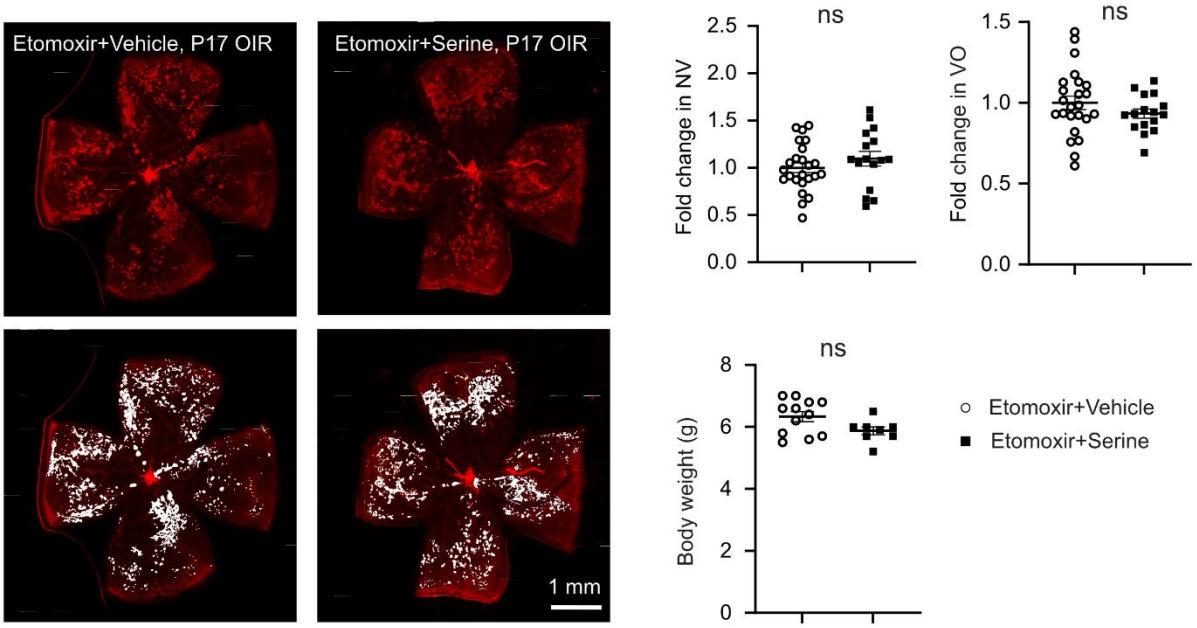

Figure S3: Blocking FAO using etomoxir attenuated serine protection against OIR. All pups were treated with etomoxir (4 mg/kg, i.p.) plus serine or vehicle from P12 to P16. At P17, retinal vaso-obliteration (VO, central area without red fluorescence) and neovascularization (NV, mid-peripheral area with high intensity of red fluorescence, highlighted in white) were examined.n = 16-24 retinas per group. Fold change was calculated relative to the average value of littermate vehicle controls. Normality (histogram, QQ-plot, Sapiro-Wilk test) and variance (F-test) were confirmed. Unpaired t-test or Welch's t-test was used to compare the groups. ns, not significant.

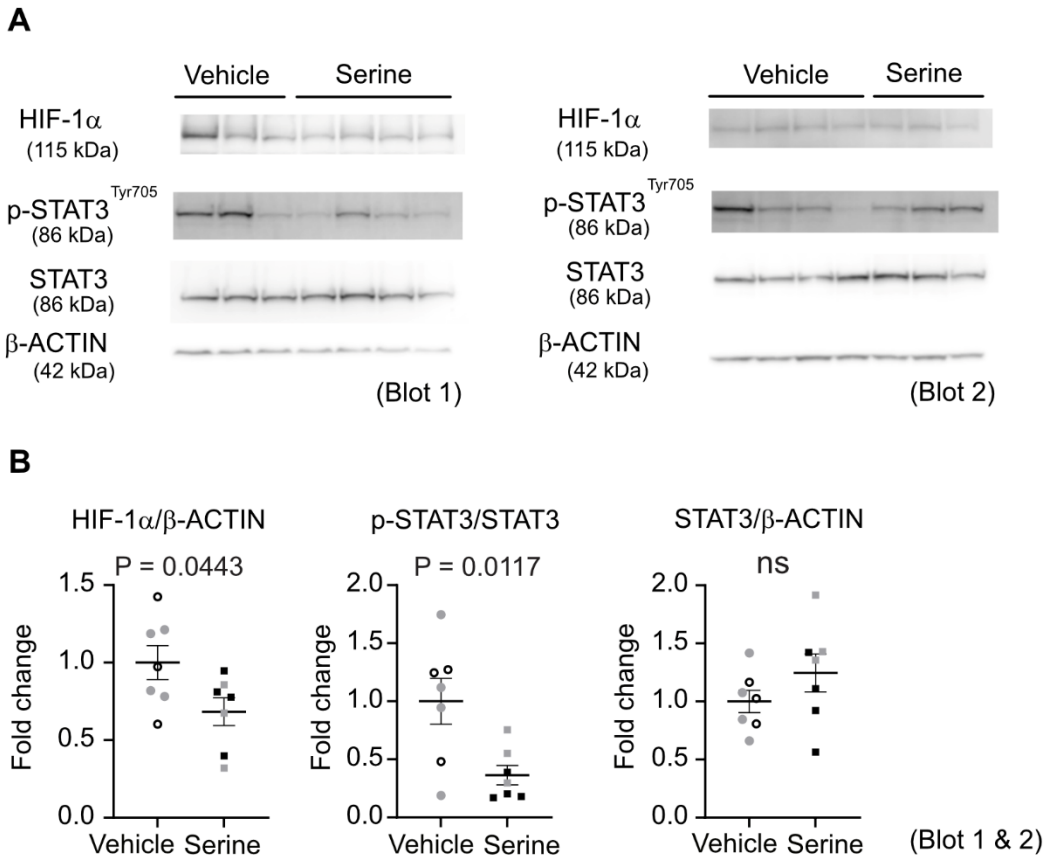

Figure S4: Serine treatment decreased pro-angiogenic signaling in OIR retinas.
(**A**) Protein levels of retinal HIF-1α, p-STAT3 (Tyr705), and STAT3 were measured in serine- vs. vehicle control-treated P17 OIR mice using western blot (two retinas were pooled per mouse as n = 1). β-ACTIN was used as an internal control. Western blot was conducted in two independent rounds using two independent mouse litters. For blot 1, n = 3 for vehicle and n = 4 for serine; for Blot 2, n = 4 for vehicle and n = 3 for serine (Vehicle, n = 7; Serine, n = 7; in total). (**B**) The fold changes in protein levels from Blot 1 (symbol in black) and Blot 2 (symbol in grey) were combined for analysis. The band intensities of target proteins were normalized to β-ACTIN levels, and the fold change was calculated relative to the average value of the vehicle controls for each blot. Normality (histogram, QQ-plot, Sapiro-Wilk test) and variance (F-test) were confirmed. Unpaired t-test was used. ns, not significant.

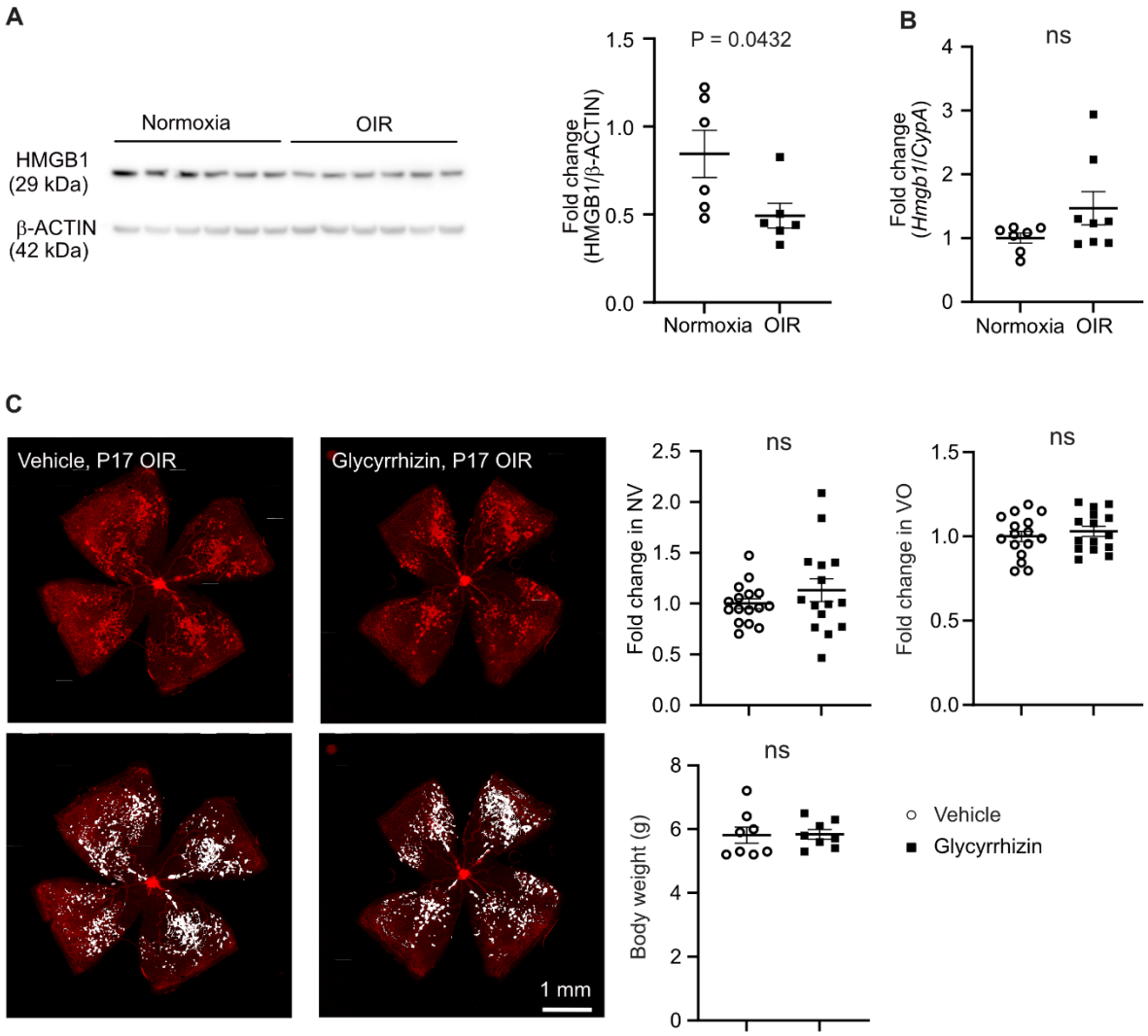

Figure S5: HMGB1 inhibitor glycyrrhizin did not affect neovascularization in OIR retinas.
(**A**) Protein levels of retinal HMGB1 were measured in P17 OIR vs. normal control (Normoxia) mice using western blot (two retinas from the same mouse were pooled as n = 1). β-ACTIN was used as an internal control. The band intensities of HMGB1 were normalized to β-ACTIN, and the fold change was calculated relative to the average value of the controls. Normality (histogram, QQ-plot, Sapiro-Wilk test) and variance (F-test) were confirmed. Unpaired t-test was used to compare the groups. Normoxia, n = 6; OIR, n = 6.
(**B**) mRNA expression levels of *Hmgb1* in P17 OIR vs. normal control (Normoxia) mice was examined with qPCR (two retinas from the same mouse were pooled as n = 1). The relative value of *Hmgb1* was divided by that of *CypA* for each sample. Fold change was calculated referring to the average relative value of control groups. Normality (histogram, QQ-plot, Sapiro-Wilk test) and variance (F-test) were confirmed. Mann-Whitney test was used to compare the groups. Normoxia, n = 7; OIR, n = 8. ns, not significant.
(**C**) OIR mouse pups were treated with HMGB1 inhibitor glycyrrhizin (25 μg/g, i.p.) or vehicle (DMSO) from P12 to P16. At P17, retinal vaso-obliteration (VO, central area without red fluorescence) and neovascularization (NV, mid-peripheral area with high intensity of red fluorescence, highlighted in white) were examined. n = 15-16 retinas per group. Fold change was calculated relative to the average value of littermate vehicle controls. Normality (histogram,

QQ-plot, Sapiro-Wilk test) and variance (F-test) were confirmed. Unpaired t-test or Welch's test was used to compare the groups. ns, not significant.

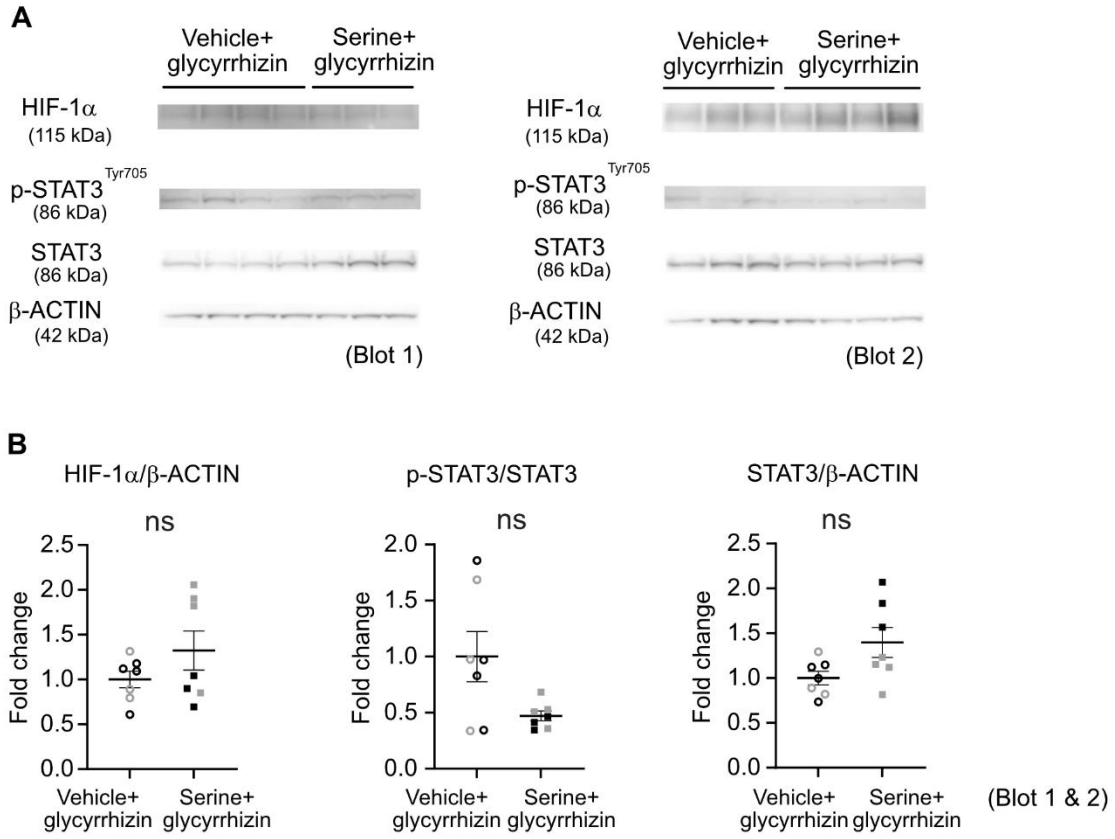

Figure S6: Blocking HMGB1 attenuated serine suppression of pro-angiogenic signaling in OIR retinas.

(**A**) Protein levels of retinal HIF-1α, p-STAT3 (Tyr705), and STAT3 were measured in serine+glycyrrhizin- vs. vehicle+glycyrrhizin- treated OIR mice using western blot (two retinas from the same mouse were pooled as n = 1). β-ACTIN was used as an internal control. Western blot was conducted in two independent rounds using two independent mouse litters. For blot 1, n = 4 for vehicle+glycyrrhizin and n = 3 for serine+glycyrrhizin; for Blot 2, n = 3 for vehicle+glycyrrhizin and n = 4 for serine+glycyrrhizin (Vehicle+glycyrrhizin, n = 7; Serine+glcyrrhizin, n = 7, in total).

(**B**) The fold changes in protein levels from Blot 1 (black symbols) and Blot 2 (grey symbols) were combined for analysis. The band intensities of target proteins were normalized to β-ACTIN, and the fold change was calculated relative to the average value of the vehicle controls on each blot. Normality (histogram, QQ-plot, Sapiro-Wilk test) and variance (F-test) were confirmed. Unpaired t-test was used. ns, not significant.

Teklad Custom Diet

TD.110839

Control AA Diet

ENVIGO

Formula	g/Kg
L-Alanine	3.5
L-Arginine HCl	12.1
L-Asparagine	6.0
L-Aspartic Acid	3.5
L-Cystine	3.5
L-Glutamic Acid	40.0
Glycine	23.3
L-Histidine HCl, monohydrate	4.5
L-Isoleucine	8.0
L-Leucine	12.0
L-Lysine HCl	18.0
L-Methionine	8.2
L-Phenylalanine	7.5
L-Proline	3.5
L-Serine	3.5
L-Threonine	8.2
L-Tryptophan	1.8
L-Tyrosine	5.0
L-Valine	8.0
Sucrose	100.0
Corn Starch	381.18
Maltodextrin	150.0
Soybean Oil	80.0
Cellulose	50.0
Mineral Mix, AIN-93M-MX (94049)	35.0
Calcium Phosphate, monobasic, monohydrate	8.2
Vitamin Mix, AIN-93-VX (94047)	13.0
Choline Bitartrate	2.5
TBHQ, antioxidant	0.02

Footnote

Amino acid defined diet designed as a control for diets with adjusted levels of amino acids. Carbohydrate provided primarily by starch.

Key Features

+ **Amino Acid Defined Diet**
+ **Rodent**
+ **Control**

Selected Nutrient Information[1]

	% by weight	% kcal from
Protein	15.4	16.4
CHO	60.6	64.5
Fat	8.0	19.2
Kcal/g	**3.8**	

[1] Calculated values

[2] Protein based on N x 6.25

Teklad Diets are designed & manufactured for research purposes only.

Key Planning Information

+ *Products are made fresh to order*
+ Store product at 4°C or lower
+ Use within 6 months (applicable to most diets)
+ Box labeled with product name, manufacturing date, and lot number
+ Replace diet at minimum once per week
  More frequent replacement may be advised
+ Lead time:
  - 2 weeks non-irradiated
  - 4 weeks irradiated

Product Specific Information

+ 1/2" Pellet or Powder (free flowing)
+ Minimum order 3 Kg
+ Irradiation not advised
  - Contact a nutritionist for recommendations

Options (Fees Will Apply)

+ Rush order (pending availability)
+ Irradiation (see Product Specific Information)
+ Vacuum packaging (1 and 2 Kg)

Speak With A Nutritionist

+ **(800) 483-5523**
+ **askanutritionist@envigo.com**

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+ **teklad@envigo.com**
+ **(800) 483-5523**

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Supplemental Table 1: Nutrient composition of serine/glycine control diet.

Teklad Custom Diet

TD.150056

Gly and Ser Deficient AA Diet, R

ENVIGO

Formula	g/Kg
L-Alanine	6.123
L-Arginine HCl	12.1
L-Asparagine	10.496
L-Aspartic Acid	6.123
L-Cystine	3.5
L-Glutamic Acid	69.976
L-Histidine HCl, monohydrate	4.5
L-Isoleucine	8.0
L-Leucine	12.0
L-Lysine HCl	18.0
L-Methionine	8.2
L-Phenylalanine	7.5
L-Proline	6.123
L-Threonine	8.2
L-Tryptophan	1.8
L-Tyrosine	5.0
L-Valine	8.0
Sucrose	100.0
Corn Starch	365.539
Maltodextrin	150.0
Soybean Oil	80.0
Cellulose	50.0
Mineral Mix, AIN-93M-MX (94049)	35.0
Calcium Phosphate, monobasic, monohydrate	8.2
Vitamin Mix, AIN-93-VX (94047)	13.0
Choline Bitartrate	2.5
TBHQ, antioxidant	0.02
Red Food Color	0.1

Footnote

Isonitrogenous modification of TD.110839 to remove glycine and serine. Color coded red.

Key Features

+ **Amino Acid Defined Diet**
+ **Isonitrogenous**
+ **Deficient Gly & Ser**
+ **Color Coded Red**

Selected Nutrient Information[1]

	% by weight	% kcal from
Protein	15.4	16.6
CHO	59.1	63.9
Fat	8.0	19.5
Kcal/g	**3.7**	

[1] Calculated values

[2] Protein based on N x 6.25

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08/28/16

Supplemental Table 2: Nutrient composition of serine/glycine deficient diet.