Supplementary Tables

Supplementary Table 1: LP/J→C57BL/6 cGVHD scoring. Each category: coat condition, skin condition, weight, posture, mobility, and vitality are individually scored and summed to achieve an overall cGVHD condition score. Scores are taken by a consistent unbiased observer with no knowledge of treatment cohorts. Scores range from 0 (healthy mouse) to 19 (mouse which has died due to cGVHD) with 18 representing the maximum score for a living mouse with cGVHD. cGVHD progression is defined as a >2 point change in overall cGVHD score from treatment baseline.

Supplementary Table 2: Drinking water administration of ibrutinib and vehicle. The average water uptake per mouse (ml) and the extrapolated daily dose (mg/kg/day) were calculated over a 7 week test period.

Supplementary Figure Legends

Supplementary Figure 1: At day 25 mice post-HSCT a total of 18 mice (from two independent experiments) were randomly assigned to ibrutinib (25mg/kg/day), 18 to vehicle, or 11 to cyclosporine (10mg/kg/day). Sclerodermatous lesions, hair loss, hunched posture, and gaunt appearance are characteristic visual indicators of cGVHD in this model. Representative visual analysis of 4 randomly selected mice at day 39 post-HSCT.

Supplementary Figure 2: H&E stained skin preparations of sclerodermatous skin lesions showing levels of dermal fibrosis, epidermal hyperplasia, serocellular crusting, erosion, and lymphohistiocytic infiltration, consistent with cGVHD.

Supplementary Figure 3: cGVHD involvement of the skin was assessed by a trained observer in a blinded fashion on a scale from 0 to 8. Cohort averages are displayed.

Supplementary Figure 4: Weekly blinded analysis of cGVHD external metrics including weight, posture, vitality, mobility, coat, and skin in all mice from two independent experiments (18 vehicle, 18 ibrutinib, and 11 cyclosporine) (Supplementary Table 1). All cGVHD scores were corrected for individual scores at the beginning of treatment (day 25). Error bars = s.e.m. *=$p<0.01$

Supplementary Figure 5: Kaplan Meier plot of cGVHD progression free survival. Progression is defined a >2 point increase in day 25 cGVHD score (Supplementary Table 1) *=$p<0.01$

Supplementary Figure 6: Kaplan Meier plot of overall survival for vehicle, ibrutinib, or cyclosporine treatment groups.

Supplementary Figure 7: Weekly bodyweight measurements for vehicle, ibrutinib, and cyclosporine treatment groups. Bodyweight was calculated to the nearest 0.1 gram at a similar time each day. Error bars = s.e.m., samples derived from two independent experiments.

Supplementary Figure 8: Representative 20X images from H&E, B220, or CD3 stained lung and kidney tissues from mice sacrificed at day 125 post-HSCT from 6 mice/group. Images were taken by a trained veterinary pathologist who was blinded to animal cohorts.

Supplementary Figure 9: Blinded pathologic analysis of H&E stained lung tissues obtained from cGVHD cohorts (18 vehicle, 18 ibrutinib, and 11 cyclosporine). Lymphohistiocytic infiltration was graded on a 0-4 scale for each animal. *=p<0.05, **=p<0.01.

Supplementary Figure 10: Blinded pathologic analysis of H&E stained kidney tissues obtained from cGVHD cohorts. Portal hepatitis and vasculitis was graded on a 0-4 scale for each animal. *=p<0.05.

Supplementary Figure 11: Weekly blinded analysis of cGVHD external metrics including weight, posture, vitality, mobility, coat, and skin in all mice from the cGVHD sustained benefit experiment (16 vehicle, 13 ibrutinib (days 25-60), 9 BM-only, 6 ibrutinib short course (days 60-72), and 7 ibrutinib continuous (days 60-72)) (Supplementary Table 1). All cGVHD scores were corrected for individual scores at the beginning of treatment (day 25). Error bars = s.e.m.

Supplementary Figure 12: Blinded pathologic analysis of H&E stained tissues obtained from cGVHD cohorts derived from the sustained benefit experiment on day 75 post HSCT. On day 60 post HSCT the ibrutinib cohort was split and 7 mice continued to receive 25mg/kg/day ibrutinib (ibrutinib continuous) while 6 mice were withdrawn from ibrutinib and placed on vehicle for the remainder of the experiment (ibrutinib short course). A) For lung tissues lymphohistiocytic infiltration was graded on a 0-4 scale for each animal. *=p<0.05. B) Blinded pathologic analysis of H&E stained kidney tissues. Portal hepatitis and vasculitis was graded on a 0-4 scale for each animal. *=p<0.05, **=p<0.01.

Supplementary Figure 13: Prophylactic treatment of cGVHD. 2-days prior to HSCT mice are randomly assigned to ibrutinib (25mg/kg/day), vehicle, or cyclosporine (10mg/kg/day) groups. Weekly blinded analysis of cGVHD external metrics including weight, posture, vitality, mobility, coat, and skin (Supplementary Table 1). All cGVHD scores were corrected for individual scores at the beginning of treatment (day -2). Error bars = s.e.m.

Supplementary Figure 14: Survival of cGVHD mice in C57BL/6→B10.BR model. Kaplan Meier plot of overall survival for bone marrow (BM) non-cGVHD mice, BM+splenocyte (S) engrafted cGVHD irrelevant vehicle treated mice, or Ibrutinib treated BM+S engrafted mice.

Supplementary Figure 15: Bodyweight of cGVHD mice in C57BL/6→B10.BR model. Bodyweight measurements for for bone marrow (BM) non-cGVHD mice, BM+splenocyte (S) engrafted cGVHD irrelevant vehicle treated mice, or Ibrutinib treated BM+S engrafted mice.

Supplementary Figure 16: PFTs were performed at day 60 or day 90 post-transplant on anesthetized animals to understand sustained benefits. Animals were artificially ventilated and resistance, elastance, and compliance were measured as parameters of distress in lung function in animals receiving $5x10^6$ splenocytes (S) in addition to bone marrow (BM). Treatment cohorts were administered vehicle or ibrutinib starting on day 28 and ending on day 56 post transplant; Error bars = s.e.m.

Supplementary Figure 17: Pooled splenic B-cells derived from healthy mice or mice with active cGVHD in either the C57BL/6->B10.BR model or the LP/J->C57BL/6 model

were interrogated for over-activated BCR pathway constituents including BTK, ERK, and IkBa.

Supplementary Materials and Methods

Therapeutic allo-HSCT models

The C57BL/6→B10.BR model has been described previously[1]. In brief, B10.BR recipients conditioned with 120mg/kg/day I.P. cyclophosphamide (Cy) on days -3 and -2 and 8.3 Gy TBI (using a ^{137}Cesium irradiator) on day -1 were engrafted with $1X10^7$ Thy1.2 depleted C57BL/6 derived bone marrow (BM) cells with (or without) $1X10^6$ allogeneic splenocytes.

Histopathological scoring

For pulmonary tissues coded pathologic analysis was conducted on H&E stained sections. Scores ranged from 0 to 4 indicating the maximum number of lymphoplasmacytic and histiocytic cellular cuffs infiltrating the surrounding airways or vasculature in 2 different 4X microscopic fields and the number of infiltrating aggregates. 0 cuffs = 0, 1 to 5 cuffs = 1, 6 to 10 cuffs and <6 aggregates = 2, 11 to 15 cuffs and <15 aggregates = 3, and >16 cuffs = 4. Limited foci of alveolar histiocytosis present with 0 cuffs were considered incidental.

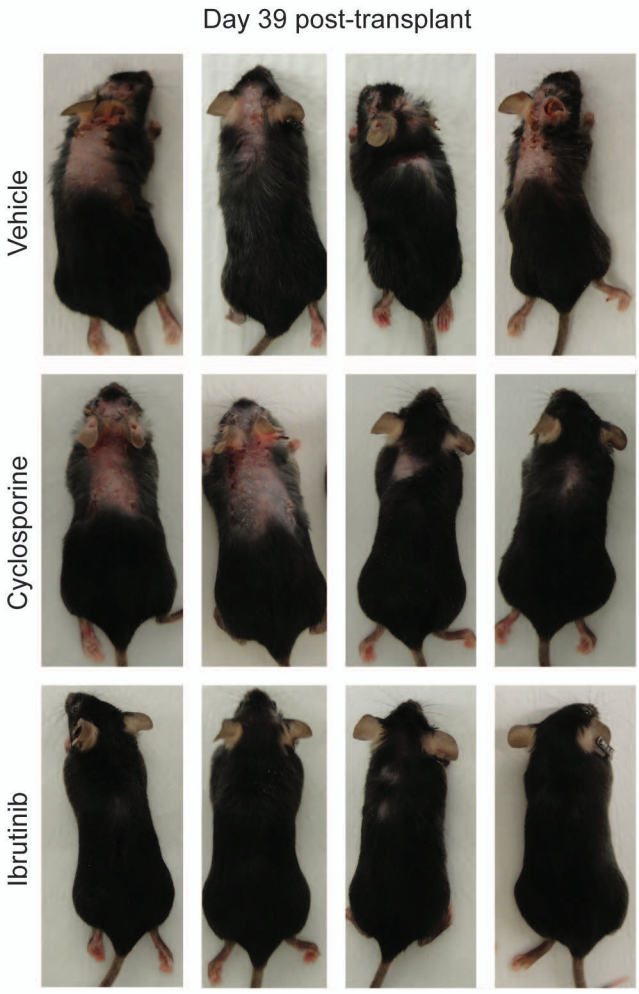
For renal H&E stained sections both perivascular lymphoplasmacytic infiltration and intratubular protein were quantified by a trained veterinary pathologist on coded specimens. Scoring ranged from 0 to 4 according to the following guidelines: No inflammatory infiltrates and hyaline eosinophilic material absent from tubular lumens =

0, Scattered foci lymphocytes and plasma cells surrounding renal vasculature or <6 tubular profiles containing hyaline eosinophilic material = 1, between 1 and 2 aggregates of inflammatory cells <10 cells in diameter or 6 to 10 tubules containing hyaline eosinophilic material = 3, between 3 and 4 foci of inflammatory cells which are up to 20 cells in diameter or between 11 and 15 tubules containing hyaline eosinophilic material = 3, 5 inflammatory cell foci or more or fewer than 5 which are >20 cells in diameter or >15 tubules containing hyaline eosinophilic material = 4.

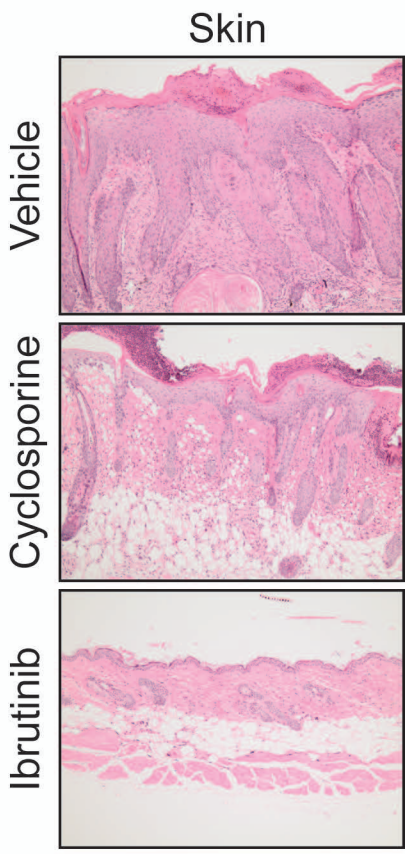
Supplementary References

1. Srinivasan M, Flynn R, Price A, et al. Donor B-cell alloantibody deposition and germinal center formation are required for the development of murine chronic GVHD and bronchiolitis obliterans. *Blood*. 2012;119(6):1570-1580.

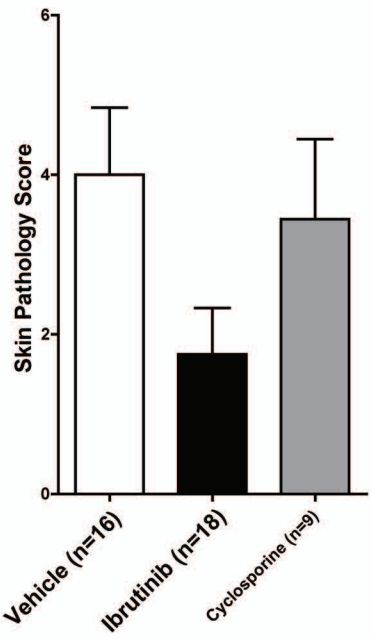
Supplementary Figure 1



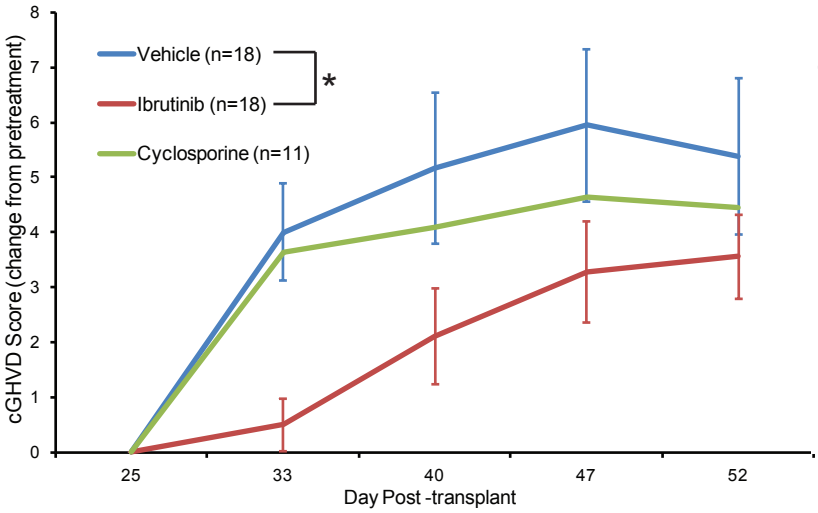
Supplementary Figure 2



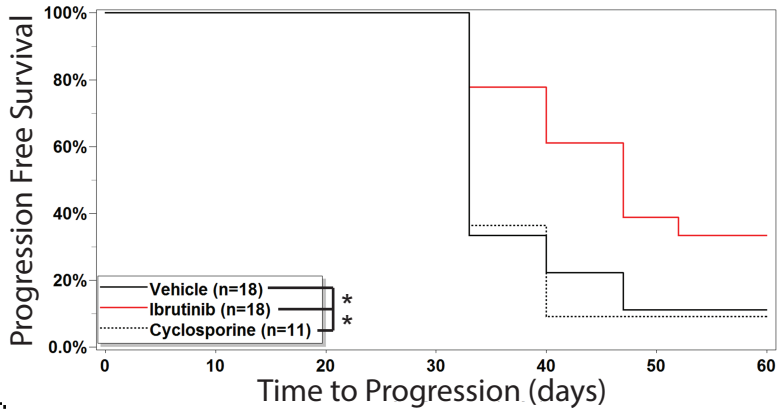
Supplementary Figure 3



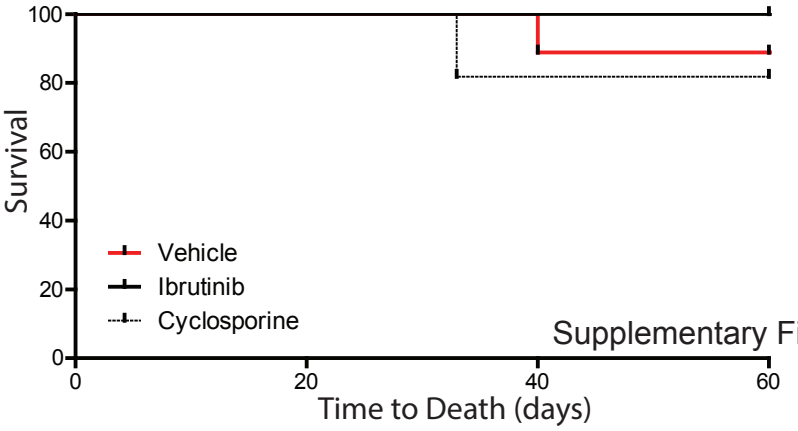
Supplementary Figure 4



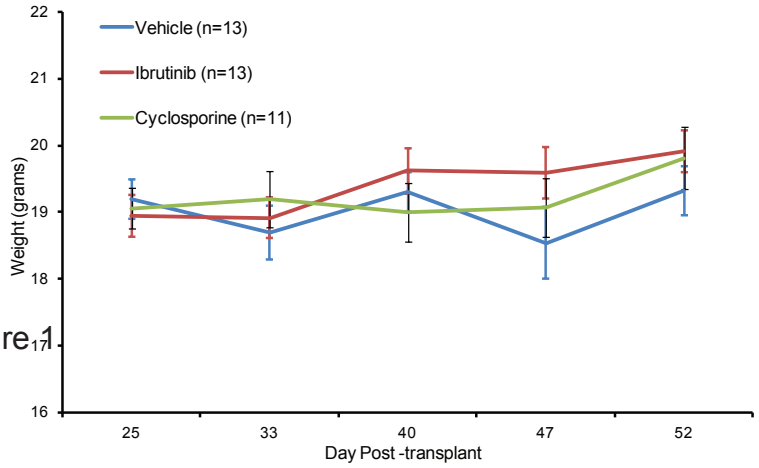
Supplementary Figure 5



Supplementary Figure 6

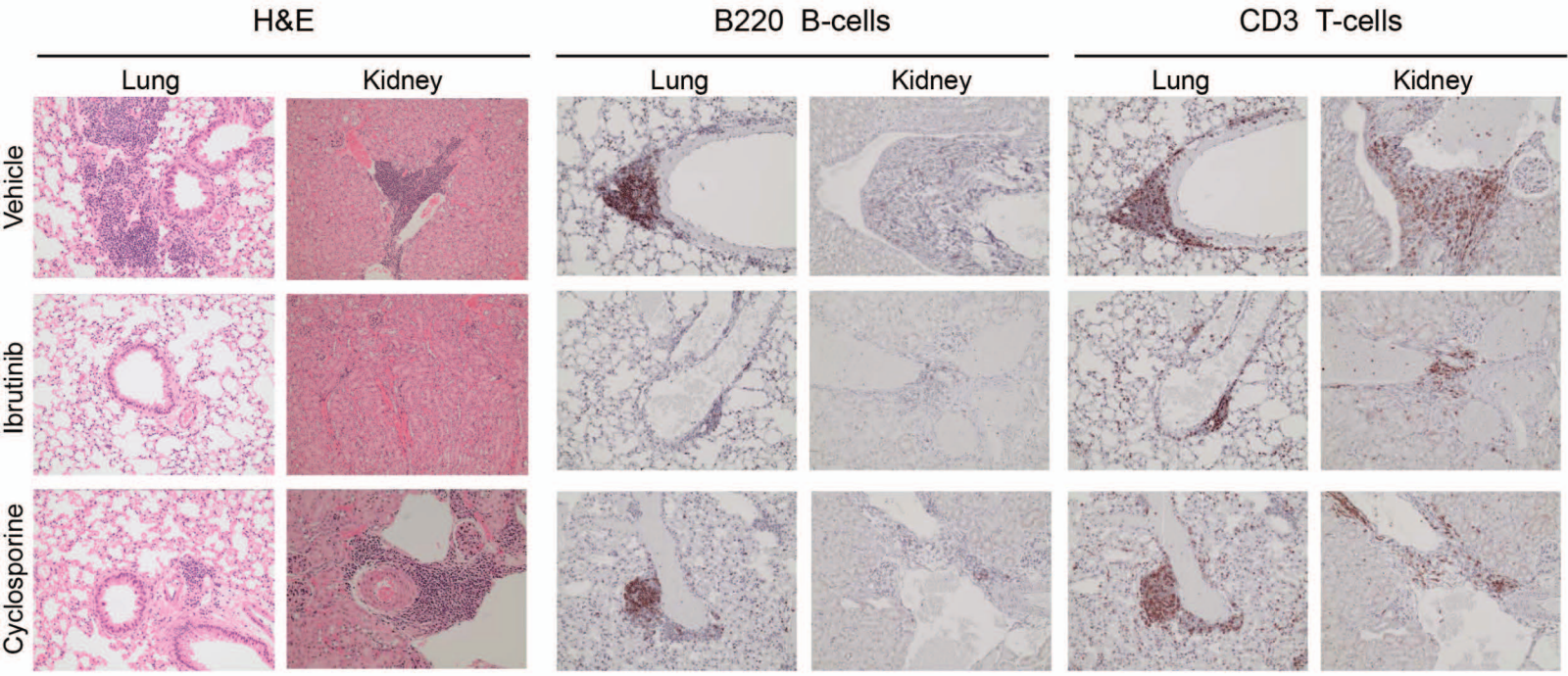


Supplementary Figure 7

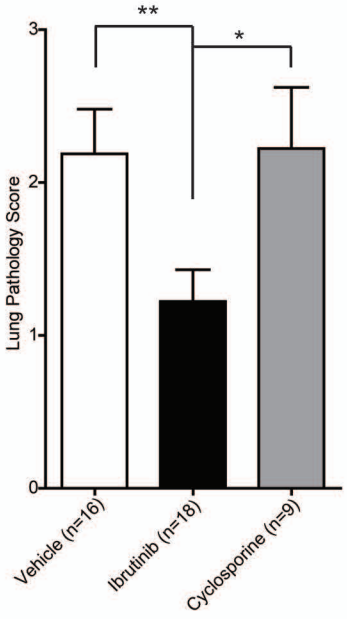


Supplementary Figure 1

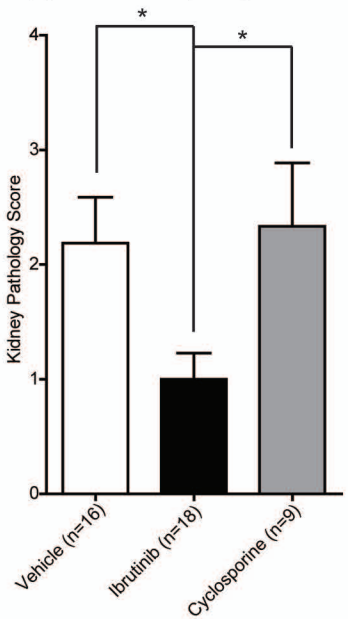
Supplementary Figure 8



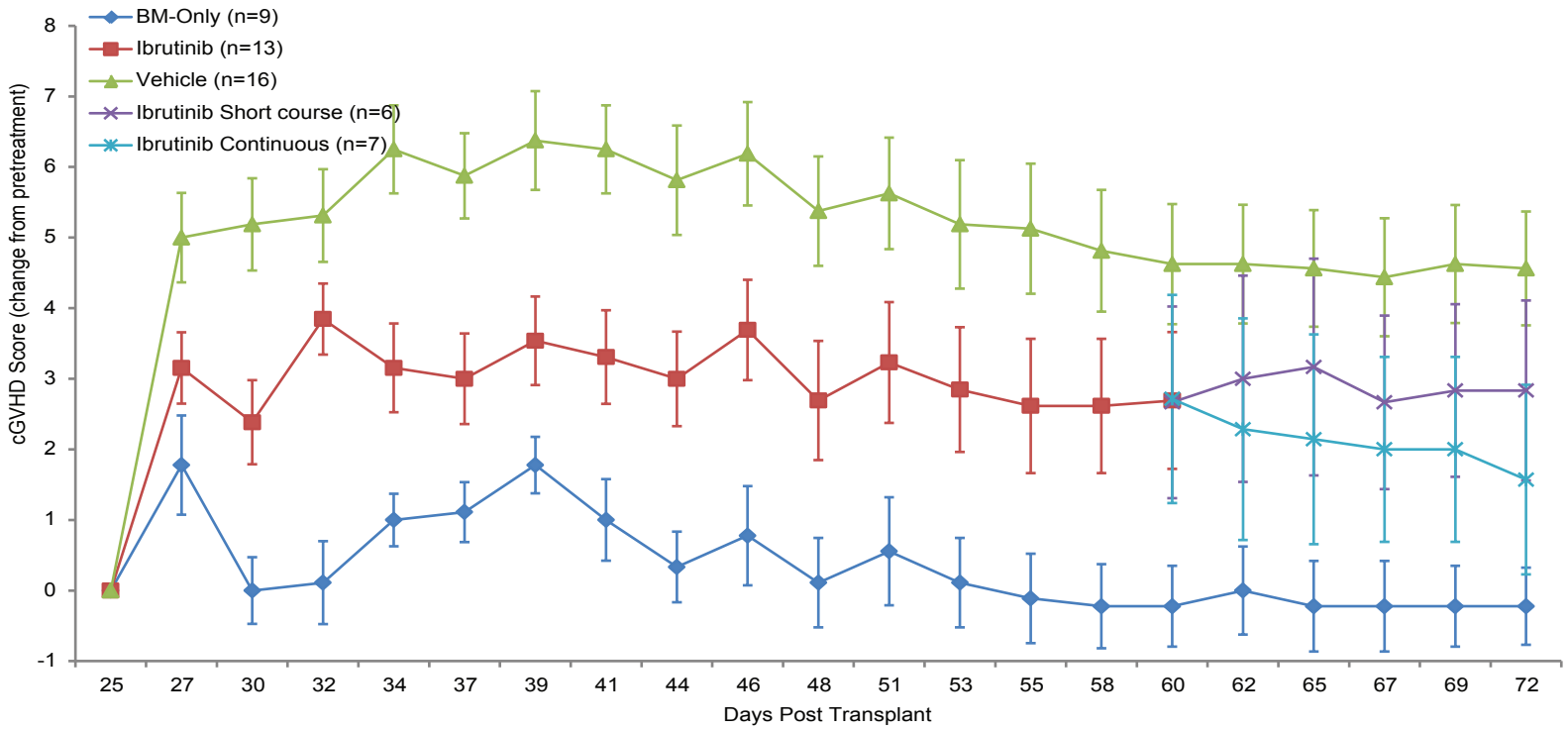
Supplementary Figure 9



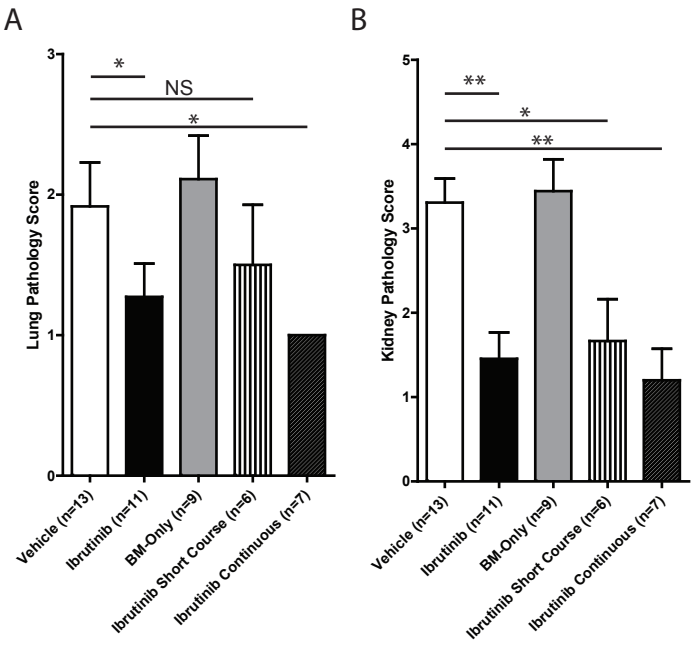
Supplementary Figure 10



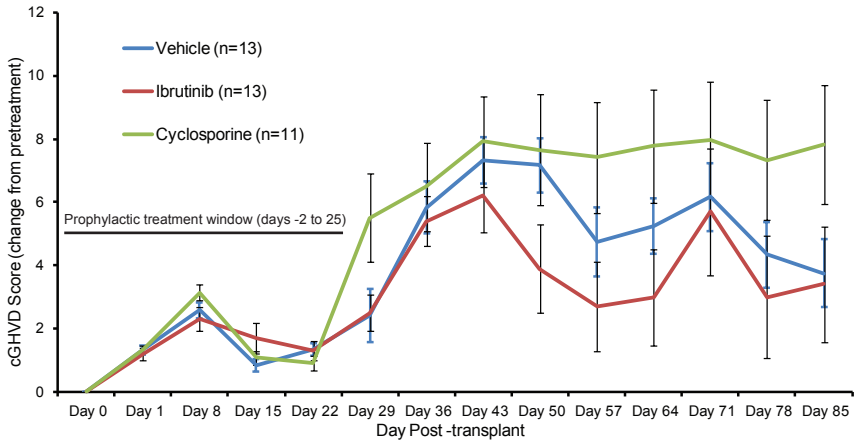
Supplementary Figure 11



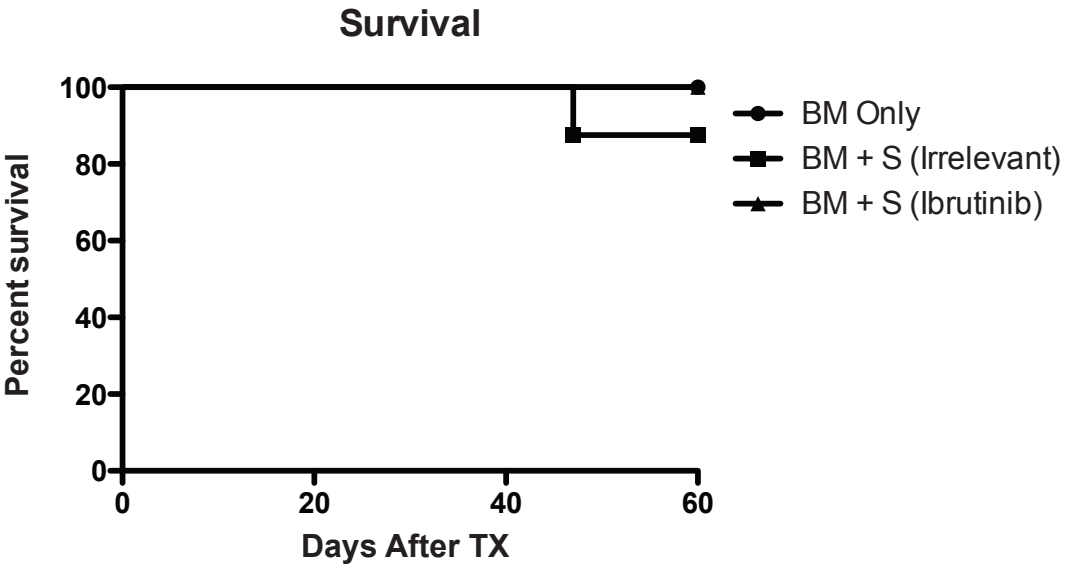
Supplementary Figure 12



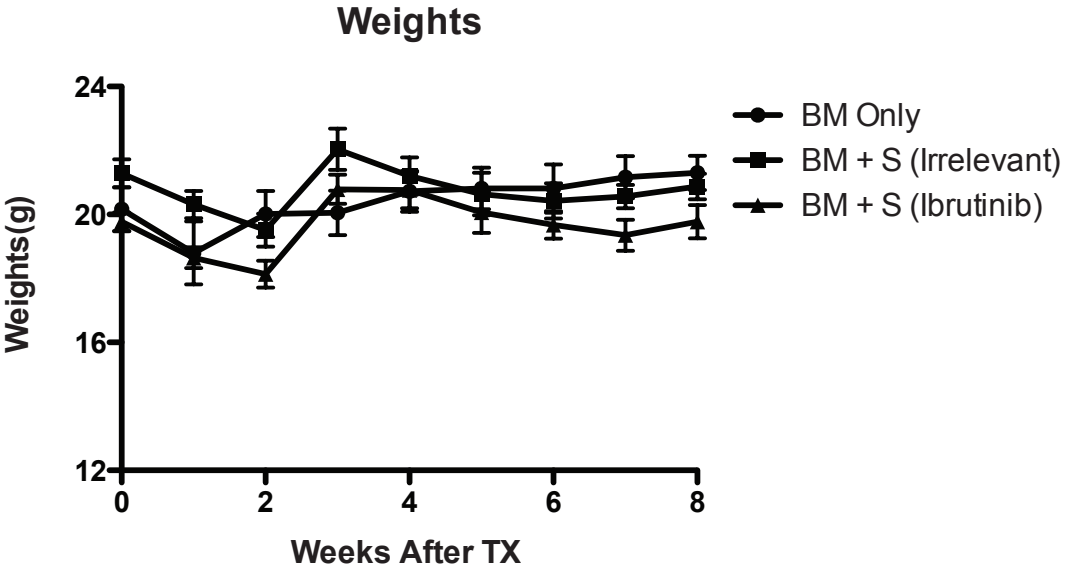
Supplementary Figure 13



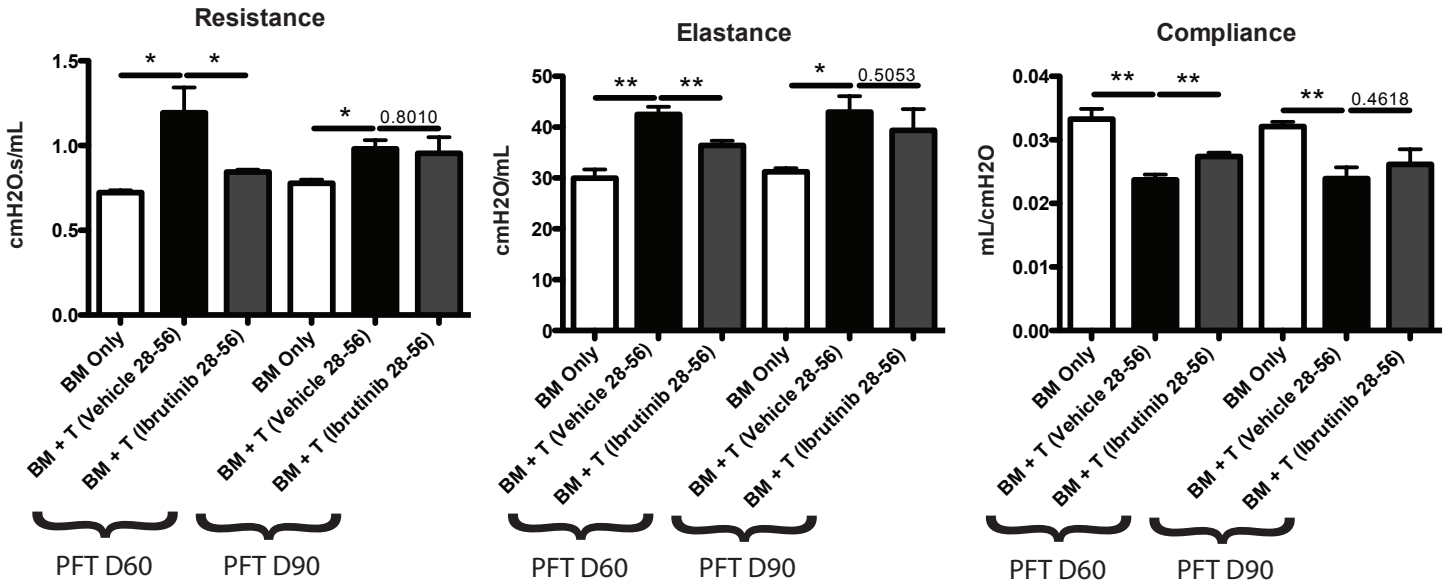
Supplementary Figure 14



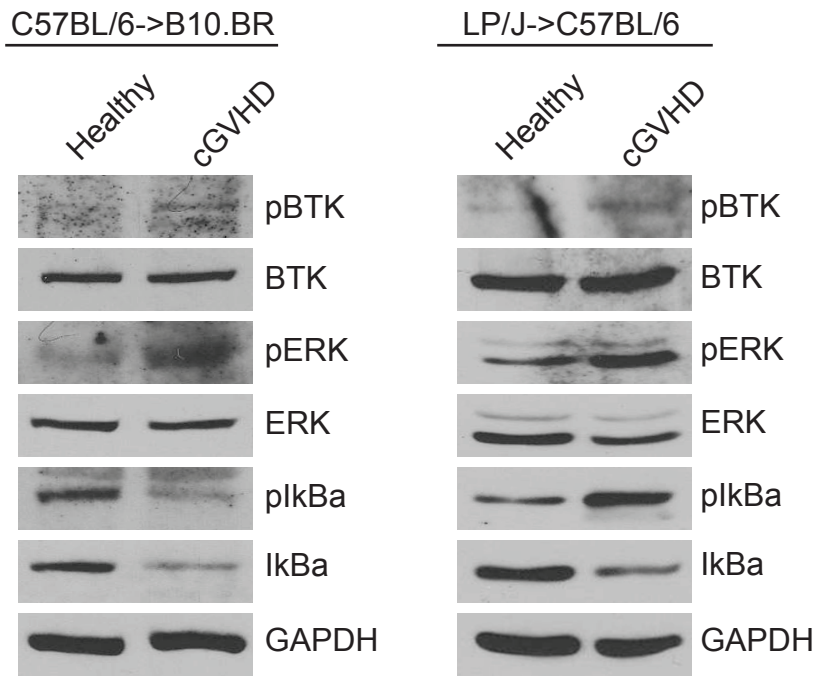
Supplementary Figure 15



Supplementary Figure 16



Supplementary Figure 17



Supplementary Table 1

Chronic Graft Versus Host Disease Scoring

Coat		Skin		Weight		Posture	
Score	Description	Score	Description	Score	Description	Score	Description
0	No hair loss	0	No sclerodermatous lesions	0	No weight loss or overall weight gain	0	No posture defect
1	Ruffled hair with a small amount of hair loss	1	Red or irritated skin lesion	1	Weigh loss <5%	1	Mild hunched posture
2	Hair loss in a single area <1cm^2	2	Skin flaking/peeling single lesion	2	Weigh loss >5% but <10%	2	Moderate hunched posture
3	Hair loss in a single area >1cm^2	3	Scabbing or bleeding in a single area	3	Weigh loss >10% but <15%	3	Severely hunched posture
4	Complete hair loss or >1 area involved	4	Scabbing or bleeding in multiple areas	4	Weigh loss >15%		

Posture		Mobility		Vitality	
Score	Description	Score	Description	Score	Description
0	No posture defect	0	Full mobility	0	Live
1	Mild hunched posture	1	Slowed gait	19	Dead
2	Moderate hunched posture	2	Slowed gait refusal to move when touched	**Instructions:** Score each category for each individual mouse. Total score is the summation of all individual scores. In the event of a dead mouse total should = 19.	
3	Severely hunched posture	3	Immobiliy when touched		

Supplementary Table 2

Treatment Group	**Week 1**	**Week 2**	**Week 3**	**Week 4**	**Week 5**	**Week 6**	**Week 7**
Vehicle Dose (mg/kg/day)	0.00	0.00	0.00	0.00	0.00	0.00	0.00
Water Volume Uptake (mL)	209.3	156.6	196.7	215.8	215.0	215.0	210.8
Ibrutinib Dose (mg/kg/day)	28.69	22.03	27.28	27.66	28.18	26.26	29.2
Water Volume Uptake (mL)	192.2	150.8	190.9	194.9	203.3	188.5	215.6